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THE COSTANTINO GORINI FOUNDATION

A foundation has been created honouring the name of Costantino GORINI, well-known dairy bacteriologist who died in September, 1950 in his native city of Milan, Italy. It was formed under the auspices of the Lombardo Institute of Sciences and Letters of Milan for the purpose of assisting research workers engaged in dairy science and technology, especially in the field of microbiology.

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The prize is 100,000 liras.

637.127.1:637.17

470. THE DISTRIBUTION OF FAT-GLOBULE SIZE IN SOW'S MILK

I. THE EFFECT OF SAMPLING AT INTERVALS THROUGHOUT MILKING

BY W. G. WHITTLESTONE

Ruakura Animal Research Station, Department of Agriculture, Hamilton, New Zealand

(With 2 Figures)

During the past year a number of studies on milk ejection in the sow has been made at this Station. The advent of the milking machine described earlier (1) has made possible the withdrawal of sow's milk under a variety of controlled conditions. These let-down studies have raised the question of the distribution of fat-globule sizes at different stages of the milking-out process. The following results show the nature of this distribution and the effects of stage of milking out. Later it is hoped to present data on the effect of stage of lactation on the fat globules in sow's milk.

METHODS

A method for the measurement of fat globules was sought which combined economy of material with the production of a permanent record of each field measured. Photographic methods were ruled out as too costly; measurement by filar micrometer or ruled ocular micrometer was found too slow and difficult. Finally, the following procedure emerged which has been found to be rapid, inexpensive and fairly easy to carry out.

APPARATUS

The microscope is fitted with a dark-ground illuminator, a 2 mm. oil-immersion objective stopped down to a numerical aperture of 0·7, a 14 × ocular and a camera lucida consisting of a prism above the ocular and a mirror. The image of a small sheet of paper on which is drawn a 5 in. circle is superimposed on the microscope field. (Because the writer requires spectacle lenses to correct for myopia a spectacle lens was inserted between the prism and the mirror in order to bring the paper into focus.) To aid in measuring, the circle (drawn in indian ink) is divided into four. The bright rings of light caused by the globules in the dark field appear quite clearly on the paper which is illuminated by a small reading lamp, the rest of the room being darkened. The globules are measured and marked off by drawing a diameter across each with a very soft, dense lead pencil. Such markings can be seen in the field and serve to 'cross off' the measured globules.

PROCEDURE

A well-mixed sample of sow's milk is diluted with a warm 10% gelatin solution at the rate of 0·3 ml. of milk to 1·0 ml. of solution. A 2 mm. platinum loopful of the warm mixture is placed on a warm slide and a square $\frac{7}{8}$ in. cover-slip dropped on. The solution

immediately runs to the edges of the cover-slip and soon solidifies. The 10% gelatin effectively stops all Brownian movement.

Measurement is effected by focusing on the top of the milk layer in each quadrant of the circle and working downwards through the several layers of globules in the normal slide. An economy in paper and time is effected by superimposing three fields on each circle. The first field is represented by horizontal lines, the second by lines at 45° to the horizontal, and the third by vertical lines.

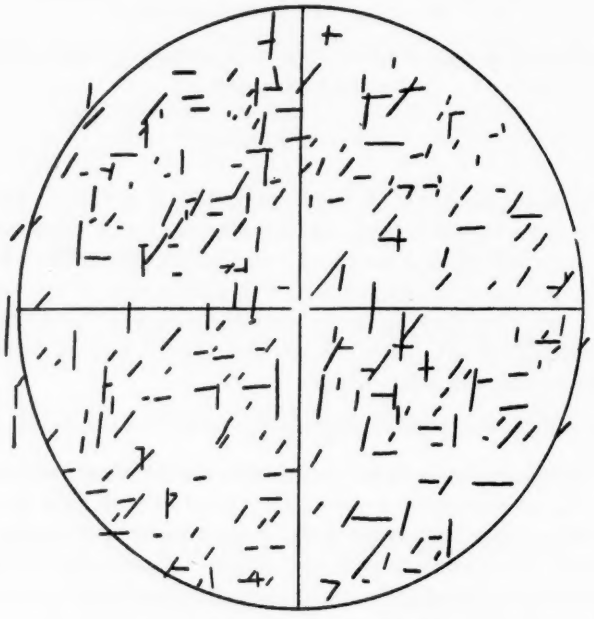

Fig. 1. Fat-globule diameter diagram.

The method is superior to the photographic one in enabling all globules to be measured in any one field instead of those in focus at the time of exposure. By using a relatively deep field there is no danger of globules being flattened by the cover-slip or of distorted distribution arising from larger globules only being counted because of their being conspicuous and together near the top of the field. A set of globule diameters is shown in Fig. 1.

The diameters are measured with a scale calibrated in microns and their distribution plotted within the size categories 0–0·5, 0·5–1, 1–1·5, 1·5–2, ..., 6·5 and above.

In Table 1 the percentage distribution of the globules is set out against diameter. The samples have been drawn in sequence, the numbers indicating the order of the milkings. When two samples only are involved the first and last have been measured. All samples represent composites from several glands and have been drawn by machine.

The sequential samples usually involve the ejection of more milk than would be drawn by a piglet at a single suckling and therefore represent normal milk plus a portion of the 'residual' milk not withdrawn under normal conditions.

Table 1. *Percentage distribution of fat-globule sizes*

Table showing percentage distribution of fat-globule diameters in samples of sow's milk drawn sequentially. Unless otherwise indicated the milk was drawn by injecting approximately 3 units of 'Pitocin' intravenously, two samples being taken during the resultant milk flow. A further similar injection was followed by one or two samples being again collected, the number depending on the effect of the hormone or the amount of milk left in the gland. In the cases marked by an asterisk the

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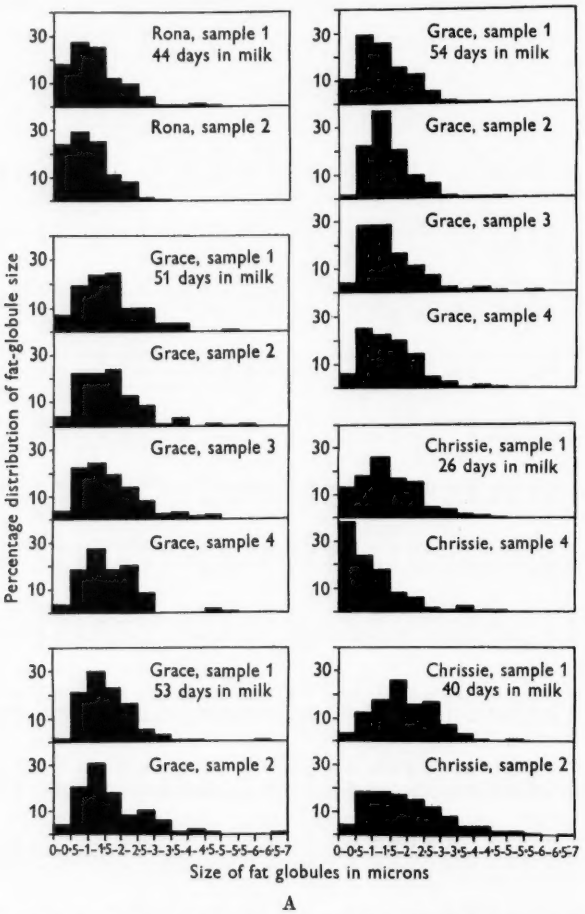
Sow	Sample no.	Days in milk	Size of fat globules in μ														No. of globules measured	% fat
			0–0·5	0·5–0·1	1–1·5	1·5–2	2–2·5	2·5–3	3–3·5	3·5–4	4–4·5	4·5–5	5–5·5	5·5–6	6–6·5	6·5		
Rebecca	1	26	2·9	21·8	15·5	25·3	15·5	8·0	7·5	2·3	—	—	0·6	0·6	—	—	174	—
	5		6·3	20·7	14·9	26·4	9·2	13·8	—	6·9	1·1	0·6	—	—	—	—	174	—
Rebecca*	1	37	2·8	13·4	17·8	29·2	13·8	13·8	4·3	2·4	1·2	0·8	0·4	—	—	—	253	—
	2		2·7	12·5	20·0	21·0	21·0	14·3	4·0	2·7	0·9	0·9	0·4	—	—	—	224	—
	3		4·6	15·3	26·5	23·5	13·8	7·7	5·1	1·0	1·5	0·5	0·5	—	—	—	196	—
	4		6·1	13·1	22·0	21·0	15·9	12·1	3·3	3·7	0·5	0·9	0·5	0·9	—	—	214	—
Rebecca	1	41	2·9	13·1	31·6	16·0	15·6	12·7	4·5	3·3	0·4	—	—	—	—	—	244	8·15
	4		1·4	14·0	30·8	23·3	16·8	9·0	2·9	0·7	0·7	0·4	—	—	—	—	279	8·07
Rebecca	1	42	4·7	25·8	35·5	15·6	11·7	3·9	2·0	0·8	—	—	—	—	—	—	256	—
	2		4·3	21·2	27·4	22·7	15·7	7·4	0·4	0·8	—	—	—	—	—	—	255	—
	3		3·0	22·5	31·0	17·5	16·0	7·5	1·5	0·5	0·5	—	—	—	—	—	200	—
	4		2·8	20·2	29·4	22·5	14·9	7·6	2·2	0·3	—	—	—	—	—	—	316	—
Agatha	1	41	25·1	24·7	28·1	9·1	8·0	1·9	—	0·8	0·4	—	—	—	—	—	263	—
	4		29·2	25·8	22·3	10·5	9·2	1·5	0·4	0·8	—	—	—	—	—	—	260	—
Agatha	1	55	2·5	23·0	33·0	18·4	12·1	8·8	1·3	0·8	—	—	—	—	—	—	239	7·35
	4		8·3	28·9	25·9	16·6	12·3	5·3	1·7	0·7	0·3	—	—	—	—	—	301	7·75
Chrissie	1	26	13·5	17·9	26·1	16·4	15·4	4·3	3·4	1·4	1·0	0·5	—	—	—	—	207	—
	4		38·7	23·8	18·1	8·2	5·8	1·6	0·8	2·5	0·8	0·8	—	—	—	—	123	—
Chrissie	1	40	3·2	12·2	17·1	25·6	15·0	16·2	6·5	2·8	0·8	—	0·4	—	—	—	246	9·39
	3		4·1	18·1	18·6	17·6	14·9	11·8	6·3	3·2	2·7	0·9	0·9	0·5	—	0·5	221	9·31
Rona	1	44	18·2	27·5	26·4	11·9	9·3	4·1	0·5	0·5	1·0	0·5	—	—	—	—	193	—
	2		24·4	29·0	25·4	11·4	8·3	1·0	0·5	—	—	—	—	—	—	—	193	—
Flika	1	32	12·8	24·4	19·4	10·0	15·6	3·3	8·9	2·8	1·7	1·1	—	—	—	—	180	—
	4		19·4	17·8	15·0	13·9	16·1	6·8	6·8	2·8	1·1	0·6	—	—	—	—	180	—
Flika	1	44	1·1	14·9	20·1	29·5	16·4	10·4	5·6	—	1·5	0·4	0·4	—	—	—	268	7·10
	3		3·0	21·7	18·7	24·3	18·3	9·7	1·9	1·5	0·7	—	—	—	—	—	267	7·35
Flika	1	48	2·8	18·6	19·8	21·4	16·3	14·7	2·4	2·0	1·6	0·4	—	—	—	—	252	9·61
	3		0·4	13·6	31·7	16·6	14·4	12·9	4·4	3·0	1·1	1·5	0·4	—	—	—	271	10·47
Grace*	1	51	7·1	19·0	23·8	24·2	9·7	3·1	3·5	—	—	0·4	—	—	—	—	227	—
	2		4·2	22·4	22·4	23·9	12·5	8·3	1·1	3·4	—	0·8	0·4	0·8	—	—	264	—
	3		3·6	22·3	24·7	19·3	13·9	7·8	2·4	3·0	1·2	1·8	—	—	—	—	166	—
	4		3·9	18·9	27·4	18·3	20·2	8·5	—	—	—	2·0	0·6	—	—	—	153	—
Grace	1	53	0·9	21·1	29·6	23·0	16·0	5·2	2·8	0·5	0·5	—	—	—	0·5	—	213	—
	4		3·9	20·2	30·2	17·1	7·9	10·1	5·7	1·3	1·8	1·3	—	—	—	0·4	228	—
Grace	1	54	10·2	29·2	25·8	15·3	12·7	5·1	0·8	0·4	0·4	—	—	—	—	—	236	6·41
	2		1·3	22·6	37·1	20·3	9·7	6·6	0·9	0·4	0·4	0·4	—	—	—	—	226	6·11
	3		3·8	28·6	28·6	16·2	10·8	7·0	1·9	1·0	1·6	0·3	—	0·3	—	—	315	5·97
	4		6·2	26·4	22·8	20·2	14·5	5·2	2·6	0·5	1·0	0·5	—	—	—	—	193	6·45
June	1	40	9·9	30·2	21·4	15·6	13·4	6·5	1·1	0·8	—	0·8	—	—	0·4	—	262	8·73
	2		8·8	20·5	23·7	19·5	10·8	8·0	4·8	2·8	0·8	—	0·4	—	—	—	249	8·58

One of the striking features of Table 1 is the wide range of fat-globule sizes. There appears to be no clear tendency for either larger or smaller globules to be drawn during the last phases of milking. It will be seen that some sows show a high percentage of globules smaller than half a micron. Such globules, which incidentally do not agglutinate as in cow's milk, do not rise to the surface under normal conditions.



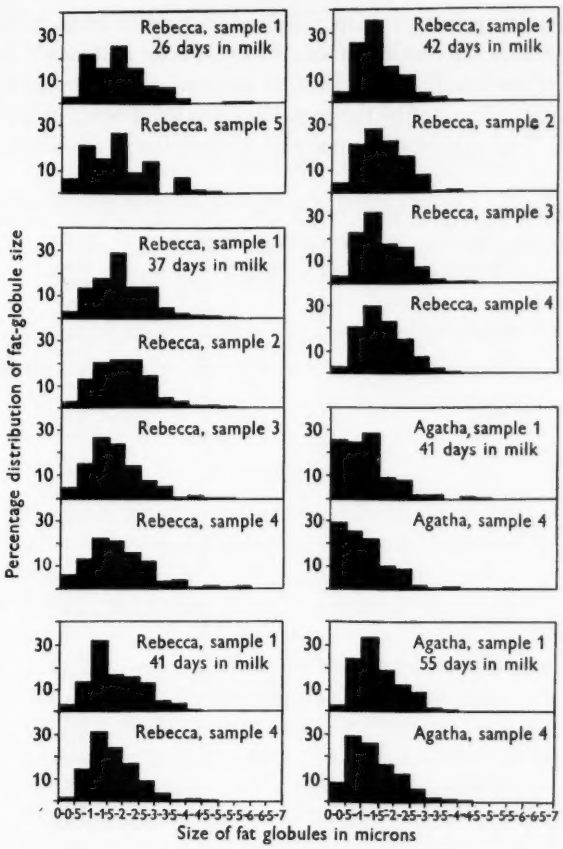
A

Fig. 2 (A–C). Fat-globule size distribution in serial samples of sow's milk.

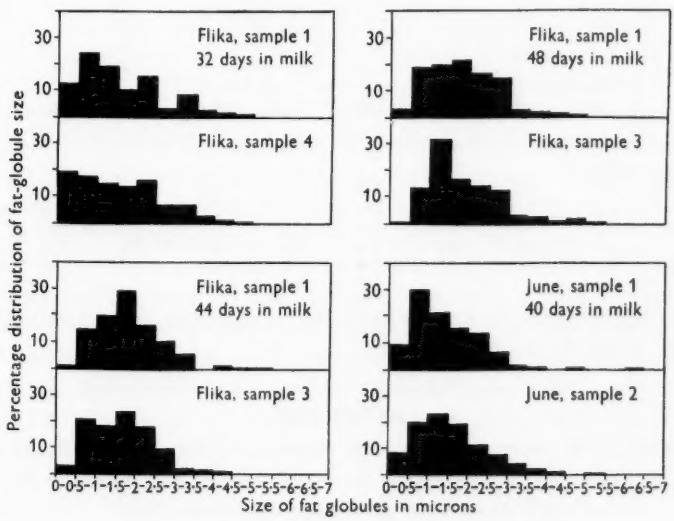
Table 2 sets out the fat percentages of sequentially drawn samples. In contrast with cow's milk, there is no marked tendency for the strippings to be richer in fat than the rest.

SUMMARY

An improved method for the determination of fat-globule size distribution has been worked out and applied to samples of sow's milk drawn sequentially. The distribution patterns for the globule sizes of sow's milk have been determined. Globules range in size from less than $\frac{1}{2}\mu$ to over 7μ. The maximum number of globules occurs at different sizes in different sows, the largest maximum size group so far recorded being $2–2{\cdot}5\mu$, the


Rebecca, sample 1
26 days in milk
Rebecca, sample 5
Rebecca, sample 1
42 days in milk
Rebecca, sample 2
Rebecca, sample 3
Rebecca, sample 4
Rebecca, sample 1
37 days in milk
Rebecca, sample 2
Rebecca, sample 3
Rebecca, sample 4
Agatha, sample 1
41 days in milk
Agatha, sample 4
Rebecca, sample 1
41 days in milk
Rebecca, sample 4
Agatha, sample 1
55 days in milk
Agatha, sample 4
Percentage distribution of fat-globule size
30
10
0·0 0·5 1·0 1·5 2·0 2·5 3·0 3·5 4·0 4·5 5·0 5·5 6·0 6·5 7·0
Size of fat globules in microns

B


Flika, sample 1
32 days in milk
Flika, sample 4
Flika, sample 1
48 days in milk
Flika, sample 3
Flika, sample 1
44 days in milk
Flika, sample 3
June, sample 1
40 days in milk
June, sample 2
Percentage distribution of fat-globule size
30
10
0·0 0·5 1·0 1·5 2·0 2·5 3·0 3·5 4·0 4·5 5·0 5·5 6·0 6·5 7·0
Size of fat globules in microns

C

smallest maximum group being 0–0·5 μ. There is no marked trend in size with stage of milking, though usually the maximum size is less for later milkings if it changes.

The strippings of sow's milk, unlike those of the cow, do not appear to be richer in fat than the earlier milk. Also unlike the cow, the globules of sow's milk do not cluster or clump (see Fig. 1). This explains the slow rate of creaming of sow's milk.

Table 2. *The fat content of sequential samples of sow's milk*

Sow	Days in milk	Sample no.	% fat
Chrissie	40	1	9·39
		2	9·82
		3	9·31
Agatha	55	1	7·35
		2	7·54
		3	7·71
		4	7·75
Flika	37	1	7·40
		2	7·46
		3	7·59
		4	7·71
		5	7·93
	44	1	7·10
		2	7·78
		3	7·35
	46	1	9·61
		2	8·15
		3	10·47
June	40	1	8·73
		2	8·58
Grace	54	1	6·41
		2	6·11
		3	5·97
		4	6·45
Rebecca	41	1	8·15
		2	8·12
		3	7·86
		4	8·07
Rona	44	1	7·78
		2	7·78
		3	8·29
		4	8·26
		5	8·22

Lucy	Days in Milk ... 49	50	51	52	53	54	55	56
Sample no.								
1	7·6	7·4	7·2	7·1	6·5	7·9	7·5	7·4
2	7·1	6·4	6·9	7·5	7·6	7·7	7·8	7·4
3	7·7	6·8	7·2	7·7	7·3	8·0	7·0	7·4
4	6·9	6·5	8·9	7·9	8·1	7·8	7·5	7·5
5	—	—	7·6	—	8·8	7·0	7·8	7·5

The writer is indebted to Mr E. D. O'Rielly for his assistance at the milking process and to Mrs D. R. Perrin for the fat analyses.

REFERENCE

(1) Smith, D. M., Whittlestone, W. G. & Allen, D. J. (1951). *J. Dairy Res.* **18**, 31.

(*MS. received for publication* 18 *October* 1951)

637.128.64

471. THE ROLE OF MICRO-ORGANISMS IN DYE-REDUCTION AND KEEPING-QUALITY TESTS

I. THE CHANGE IN DOMINANT BACTERIAL FLORA DURING INCUBATION AT 37·5 AND 22° C. AND THE PART PLAYED BY STAPHYLOCOCCI AND THE *COLI-AEROGENES* GROUP

By ELLEN I. GARVIE and A. ROWLANDS

National Institute for Research in Dairying, University of Reading

(With 4 Figures)

INTRODUCTION

Recent work by Rowlands, Barkworth, Hosking & Kempthorne (1) questioned the validity of the methylene blue and resazurin reduction tests at 37·5 and 18° C. as measures of the actual keeping quality of milk. They used the clot-on-boiling test as an objective measure of the end-point of keeping quality for milk samples incubated at 18° C. For a series of samples a fair agreement was found between the end-points of the dye tests at 37·5 and 18° C. and keeping quality at 18° C., but, for individual samples, keeping quality could not be accurately forecast from the results of the dye tests.

The present work is an attempt to explain the discrepancies between the results for the keeping-quality and dye-reduction tests. There are several papers dealing with the bacteriological flora of milk at the time of methylene-blue reduction (Wilson (2), Jensen (3), Davis & Lines (4), Hobbs (5), Thomé (6)), but no similar work has appeared dealing with the flora of raw milk at the end-point of a keeping-quality test after incubation at temperatures approximating to those of actual storage by the consumer. It is clear from the observations of Wilson (2) that differences do occur between the dominant flora present at the time of reduction of methylene blue after incubation at 37·5 and 17·5° C. At 37·5° C. micrococci and staphylococci were the most frequently isolated, whereas at 17·5° C. achromobacteria and *Pseudomonas fluorescens* were the most common.

In samples incubated at 37·5° C., particularly when reduction time was long, Wilson (2) and Hobbs (5) found staphylococci to predominate, and because of their possible role in food poisoning Wilson expressed alarm at the frequency with which they became dominant at this temperature, particularly in certified milk. However, staphylococci were not isolated at the time of reduction of the same samples incubated at 30 or 17·5° C., which suggests that this group of organisms is unimportant in milk spoilage. Wilson (2), Hobbs (5) and Thomé (6) found numbers of the *coli-aerogenes* group in a proportion of their samples at the time of reduction of methylene blue at 37·5° C., but Jensen (3) regards them as unimportant. Early work by Barkworth, Mattick, Taylor & Williams (7), Barkworth, Meanwell & Taylor (8), Barkworth (9) and Malcolm (10) laid stress on the apparent reduction in keeping quality with increasing numbers of the *coli-aerogenes* group. In the present study special attention has been given to the staphylococci and members of the *coli-aerogenes* group.

EXPERIMENTAL METHODS

Source and storage of samples. The raw-milk samples were taken from nineteen local farms immediately on completion of the evening milking. In all, forty-one samples covering both winter and summer periods were examined. All the samples reached the laboratory within 2 hr. of sampling and were then stored at 14° C. overnight.

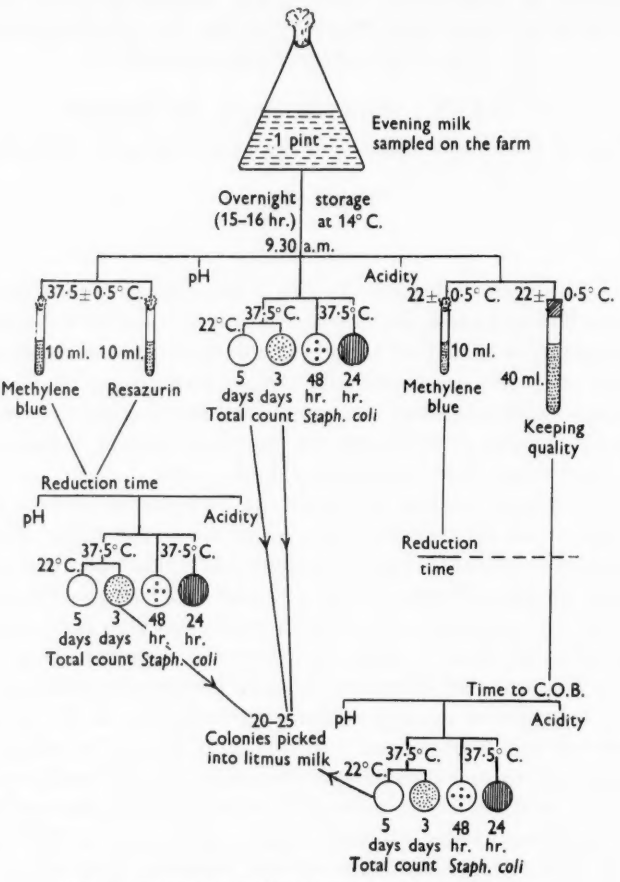


Fig. 1. To show the method of treatment of milk samples.

Examination of the samples. The treatment of the samples is represented diagrammatically in Fig. 1. Testing was begun between 9 and 10 a.m. on the day following sampling, hereafter called initial, and all the samples were examined by the methylene-blue and resazurin reduction tests at 37·5 ± 0·5° C. and by the methylene-blue and keeping-quality (clot-on-boiling) tests at 22 ± 0·5° C., using the method of Rowlands *et al.* (1), except that for examination by the clot-on-boiling test the samples were incubated in 8 × 1 in. Pyrex boiling tubes closed with rubber stoppers (Garvie & Rowlands (11)).

Enumeration of staphylococci, members of the coli-aerogenes *group and total bacteria*

Differential media were used for the enumeration of the staphylococci and *coli-aerogenes* bacteria, plates being inoculated initially, at the time of reduction of methylene blue at 37·5° C. and at the time of the end-point of the clot-on-boiling test at 22° C. At these same times an estimation of the total colony count was made, using yeastrel milk agar (Ministry of Health (12)).

For the enumeration of the staphylococci the drop-plate technique of Miles & Misra (13) was adopted, using the following modification of the mannitol salt agar of Chapman (14, 15): 1·0% lemco, 1·0% peptone, 8·0% NaCl, 2·0% agar, 0·0025% phenol red, pH 8·6.

The medium was sterilized in 100 ml. quantities. Immediately before use 5 ml. of a 10% solution of mannitol sterilized by Seitz filtration was added to the melted and cooled medium.

The plates were counted after incubation at 37·5° C. for 48 hr. A further count of orange colonies was made after exposure on the laboratory bench in the light for 24 hr.

For the enumeration of the *coli-aerogenes* group deep plates were prepared using violet-red bile agar (Bartram & Black (16)) and counted after incubation at 37·5° C. for 24 hr.

For the total colony count deep plates were prepared, using yeastrel milk agar. For thirteen samples duplicate plates were prepared for incubation at 37·5° C. for 3 days and at 22° C. for 5 days; for the remaining twenty-eight samples the plates were incubated at 30° C. for 3 days.

Examination of the predominant bacterial flora. A detailed study was made of the flora of twelve samples. Initially, poured plates were prepared in duplicate, one set being incubated at 37·5° C. and the other at 22° C. At the time of reduction of methylene blue at 37·5° C. one set of plates was prepared and incubated at this temperature for 3 days. At the time of the end-point of the clot-on-boiling test a single set of plates was also prepared but incubated at 22° C. for 5 days. There were, therefore, four sets of cultures from each sample; from plates prepared from the initial milk and incubated at 22 and 37·5° C., from plates prepared at the time of dye reduction and incubated at 37·5° C., and from plates prepared at the end-point of the clot-on-boiling test and incubated at 22° C.

RESULTS AND DISCUSSION

The incidence of staphylococci as indicated by plating on a differential medium

The distribution of staphylococci in the samples is given in Tables 1, 2 and 3. Initially staphylococci were found in most of the samples examined, although in these samples they constituted a relatively small proportion of the total flora. In twenty-six of the thirty-eight samples staphylococci formed less than 1% of the total flora; in only four samples was the proportion greater than 10%. None of the samples examined was free from staphylococci, although in eleven samples none was recorded initially on the lowest dilution plated. Bearing in mind the prevalence of staphylococcal udder infections (Minett (17), Shattock & Mattick (18)) these findings are not surprising.

At the time of dye reduction at 37·5° C. the count of staphylococci showed an increase over the initial count in all but one sample, and with three exceptions an increase in the percentage of staphylococci. Only nine samples contained less than 1% staphylococci and eight of these had a dye reduction time of less than 5 hr., while ten samples contained

Table 1. (*a*) *Staphylococci and* coli-aerogenes *bacteria in samples of raw milk*

No. of sample	Methylene-blue reduction time (37·5° C.)	Time of C.O.B. test end-point (22° C.)	Initial milk: Total bacteria 22° C.	Total bacteria 37·5° C.	Total bacteria 30° C.	Staphylococci Count	Staphylococci %	*Coli-aerogenes* bacteria Count	*Coli-aerogenes* bacteria %
10 (28/9)	1	11	—	—	2,247,000	4,000	0·18	+100,000	+4·00
10 (3/7)	1½	14	—	—	888,000	6,280	0·73	15,800	1·69
4 (2/5)	2	17	—	—	457,000	98	0·02	4,000	0·88
31	2½	10½	2,790,000	1,710,000	—	350	0·02	115,000	6·73
30	2½	13	3,190,000	1,130,000	—	3,980	0·35	25,000	2·21
8 (2/5)	2½	20	—	—	314,000	100	0·03	109,600	34·93
2 (28/9)	2½	26	—	—	1,724,000	970	0·06	180	0·01
2 (2/5)	3½	11	—	—	1,584,000	<76	<0·01	1,690	0·11
32	3½* 5½	17	434,000	300,000	—	<76	<0·03	0	0·00
34	3½	20	143,000	154,000	—	290	0·19	330	0·21
10 (23/11)	4	20	—	—	564,000	190	0·03	1,810	0·32
33	4½	20	44,800	30,000	—	780	2·60	730	2·43
8 (15/2)	4½	20	—	—	232,000	<76	<0·03	9,700	4·18
5 (28/9)	5	17	—	—	59,000	4,900	10·96	2,280	4·98
29	5	23	184,000	112,000	—	3,700	3·30	200	0·18
5 (23/11)	5½	20	—	—	78,000	690	0·87	10,400	13·33
35	6	17	52,000	43,000	—	162	0·38	436	1·01
1 (3/7)	6	20	—	—	42,000	<76	<0·18	28,300	67·38
6 (3/7)	6	23	—	—	68,000	5,800	8·53	700	1·03
11 (15/2)	6½	20	—	—	89,000	190	0·21	400	0·45
28	6½	32	8,300	4,550	—	<76	<1·69	2	0·04
7 (15/2)	6½	50	—	—	4,150	290	6·9	0	0·00
11 (23/11)	7	20	—	—	31,400	4,600	14·65	1,250	3·98
25	7	21½	72,700	20,800	—	<76	<0·36	—	—
9 (28/9)	7	29	—	—	39,000	1,850	4·87	10	0·03
24	7	32	1,370	1,600	—	—	—	—	—
7 (2/5)	7	38	—	—	2,720	195	7·15	0	0·00
26	7	42	2,130	2,060	—	—	—	—	—
4 (23/11)	7½	20	—	—	398,000	1,000	0·25	500	0·13
1 (23/11)	7½	23	—	—	69,000	100	0·15	260	0·38
6 (28/9)	7½	23	—	—	562,000	2,200	0·39	1,010	0·18
3 (15/2)	7½	32	—	—	2,780	190	6·83	50	1·80
2 (15/2)	7½	41	—	—	2,270	290	12·78	90	3·90
9 (3/7)	7½	41	—	—	2,880	120	4·16	150	5·21
27	7½	46	1,430	2,080	—	<76	<3·66	—	—
9 (15/2)	7½	50	—	—	1,200	<76	<6·33	20	1·67
6 (2/5)	8	38	—	—	10,700	<76	<0·71	0	0·00
12 (2/5)	8	50	—	—	2,000	<76	<3·80	0	0·00
3 (28/9)	8½	39½	—	—	1,200	780	65·00	10	0·83
12 (23/11)	8½	44	—	—	1,020	<76	<7·45	—	—

* Resazurin reduction time. — No estimate made of bacteria present.

Table 1. (*b*) *Staphylococci and* coli-aerogenes *bacteria in samples of raw milk*

No. of sample	Methylene-blue reduction time (37·5° C.)	Time of C.O.B. test end-point (22° C.)	Time of dye reduction: Total bacteria 22° C.	Total bacteria 37·5° C.	Total bacteria 30° C.	Staphylococci Count	Staphylococci %	*Coli-aerogenes* bacteria Count	*Coli-aerogenes* bacteria %
10 (28/9)	1	11	—	—	2,247,000	4,000	0·18	+100,000	+4·00
10 (3/7)	1½	14	—	—	888,000	6,280	0·73	15,800	1·69
4 (2/5)	2	17	—	—	457,000	98	0·02	4,000	0·88
31	2½	10½	2,790,000	1,710,000	—	350	0·02	115,000	6·73
30	2½	13	3,190,000	1,130,000	—	3,980	0·35	25,000	2·21
8 (2/5)	2½	20	—	—	314,000	100	0·03	109,600	34·93
2 (28/9)	2½	26	—	—	1,724,000	970	0·06	180	0·01
2 (2/5)	3½	11	—	—	1,584,000	<76	<0·01	1,690	0·11
32	3½* 5½	17	434,000	300,000	—	<76	<0·03	0	0·00
34	3½	20	143,000	154,000	—	290	0·19	330	0·21
10 (23/11)	4	20	—	—	564,000	190	0·03	1,810	0·32
33	4½	20	44,800	30,000	—	780	2·60	730	2·43
8 (15/2)	4½	20	—	—	232,000	<76	<0·03	9,700	4·18
5 (28/9)	5	17	—	—	59,000	4,900	10·96	2,280	4·98
29	5	23	184,000	112,000	—	3,700	3·30	200	0·18
5 (23/11)	5½	20	—	—	78,000	690	0·87	10,400	13·33
35	6	17	52,000	43,000	—	162	0·38	436	1·01
1 (3/7)	6	20	—	—	42,000	<76	<0·18	28,300	67·38
6 (3/7)	6	23	—	—	68,000	5,800	8·53	700	1·03
11 (15/2)	6½	20	—	—	89,000	190	0·21	400	0·45
28	6½	32	8,300	4,550	—	<76	<1·69	2	0·04
7 (15/2)	6½	50	—	—	4,150	290	6·9	0	0·00
11 (23/11)	7	20	—	—	31,400	4,600	14·65	1,250	3·98
25	7	21½	72,700	20,800	—	<76	<0·36	—	—
9 (28/9)	7	29	—	—	39,000	1,850	4·87	10	0·03
24	7	32	1,370	1,600	—	—	—	—	—
7 (2/5)	7	38	—	—	2,720	195	7·15	0	0·00
26	7	42	2,130	2,060	—	—	—	—	—
4 (23/11)	7½	20	—	—	398,000	1,000	0·25	500	0·13
1 (23/11)	7½	23	—	—	69,000	100	0·15	260	0·38
6 (28/9)	7½	23	—	—	562,000	2,200	0·39	1,010	0·18
3 (15/2)	7½	32	—	—	2,780	190	6·83	50	1·80
2 (15/2)	7½	41	—	—	2,270	290	12·78	90	3·90
9 (3/7)	7½	41	—	—	2,880	120	4·16	150	5·21
27	7½	46	1,430	2,080	—	<76	<3·66	—	—
9 (15/2)	7½	50	—	—	1,200	<76	<6·33	20	1·67
6 (2/5)	8	38	—	—	10,700	<76	<0·71	0	0·00
12 (2/5)	8	50	—	—	2,000	<76	<3·80	0	0·00
3 (28/9)	8½	39½	—	—	1,200	780	65·00	10	0·83
12 (23/11)	8½	44	—	—	1,020	<76	<7·45	—	—

* Resazurin reduction time. — No estimate made of bacteria present.

12 (23/11)	8½	44	—	—	1,020	<76	<7·45	—	—

* Resazurin reduction time. — No estimate made of bacteria present.

Table 1. *(b) Staphylococci and* coli-aerogenes *bacteria in samples of raw milk*

No. of sample	Methylene-blue reduction time (37·5° C.)	Time of C.O.B. test end-point (22° C.)	Time of dye reduction: Total bacteria 22° C.	Total bacteria 37·5° C.	Total bacteria 30° C.	Staphylococci Count	Staphylococci %	*Coli-aerogenes* bacteria Count	
10 (28/9)	1	11	—	—	5,700,000	6,000	0·11	1,660,000	29·12
10 (3/7)	1½	14	—	—	2,300,000	—	—	19,400	0·85
4 (2/5)	2	17	—	—	5,210,000	1,390	0·03	11,200	0·22
31	2½	10½	8,800,000	9,300,000	—	6,190	0·07	1,820,000	19·55
30	2½	13	2,990,000	2,190,000	—	47,200	2·16	80,300	3·66
8 (2/5)	2½	20	—	—	2,420,000	690	0·03	930,000	38·43
2 (28/9)	2½	26	—	—	1,440,000	6,000	0·42	100	0·01
2 (2/5)	3½	11	—	—	17,700,000	318,000	1·73	12,900	0·07
32	3½*	17	17,200,000	15,850,000	—	48,500	0·36	1,760	0·01
	5½		10,500,000	11,200,000		1,360,000	12·13	5,350	0·05
34	3½	20	1,860,000	2,490,000	—	28,000	1·13	8,330	0·33
10 (23/11)	4	20	—	—	4,450,000	12,900	0·29	1,000	0·02
33	4½	20	7,900,000	7,050,000	—	3,680,000	64·75	101,300	1·78
8 (15/2)	4½	20	—	—	6,700,000	76	<0·01	2,050,000	30·60
5 (28/9)	5	17	—	—	10,000,000	149,000	1·49	2,780,000	27·80
29	5	23	760,000	940,000	—	170,000	18·08	196,000	20·85
5 (23/11)	5½	20	—	—	17,600,000	421,000	2·39	830,000	4·72
35	6	17	12,800,000	11,800,000	—	2,860,000	24·25	3,630	0·03
1 (3/7)	6	20	—	—	5,300,000	—	—	2,980,000	56·23
6 (3/7)	6	23	—	—	14,400,000	—	—	200	0·01
11 (15/2)	6½	20	—	—	28,900,000	8,550,000	29·59	37,000	0·13
28	6½	32	8,300,000	8,600,000	—	—	—	840,000	9·77
7 (15/2)	6½	50	—	—	12,000,000	—	—	500	0·01
11 (23/11)	7	20	—	—	3,350,000	317,000	9·46	29,600	0·87
25	7	21½	2,910,000	3,590,000	—	468,000	13·04	—	—
9 (28/9)	7	29	—	—	2,980,000	5,225,000	100·00	27,000	0·91
24	7	32	2,340,000	2,650,000	—	2,670,000	100·00	—	—
7 (2/5)	7	38	—	—	11,500,000	10,490,000	100·00	12,100	0·11
26	7	42	1,530,000	2,480,000	—	1,550,000	62·50	—	—
4 (23/11)	7½	20	—	—	7,300,000	637,000	8·73	4,400	0·06
1 (23/11)	7½	23	—	—	10,000,000	13,580,000	100·00	4,100	0·04
6 (28/9)	7½	23	—	—	19,700,000	1,950,000	9·91	12,480,000	63·35
3 (15/2)	7½	32	—	—	23,900,000	9,060,000	37·91	2,400,000	10·05
2 (15/2)	7½	41	—	—	10,900,000	14,350,000	100·00	2,100	0·02
9 (3/7)	7½	41	—	—	9,600,000	—	—	100	0·01
27	7½	46	5,280,000	5,450,000	—	2,510,000	46·05	—	—
9 (15/2)	7½	50	—	—	38,400,000	44,750,000	100·00	400	0·01
6 (2/5)	8	38	—	—	4,990,000	3,830,000	76·75	660,000	13·23
12 (2/5)	8	50	—	—	7,520,000	13,700,000	100·00	4,800	0·06
3 (28/9)	8½	39½	—	—	121,600,000	556,000	0·46	1,260,000	1·04
12 (23/11)	8½	44	—	—	39,500,000	6,560,000	16·60	11,000,000	27·85

* Resazurin reduction time. — No estimate made of bacteria present.

Table 1. *(c) Staphylococci and* coli-aerogenes *bacteria in samples of raw milk*

No. of sample	Methylene-blue reduction time (37·5° C.)	Time of C.O.B. test end-point (22° C.)	Time of clot-on-boiling: Total bacteria 22° C.	Total bacteria 37·5° C.	Total bacteria 30° C.	Staphylococci Count	Staphylococci %	*Coli-aerogenes* bacteria Count	*Coli-aerogenes* bacteria %
10 (28/9)	1	11	—	—	770,000,000	1,300	<0·01	35,700,000	4·55
10 (3/7)	1½	14	—	—	340,000,000	61,000	0·02	450,000	0·13
4 (2/5)	2	17	—	—	206,000,000	690	<0·01	32,000	0·02
31	2½	10½	440,000,000	207,000,000	—	690	<0·01	430,000	0·21
30	2½	13	374,000,000	63,600,000	—	21,800	0·03	6,400,000	1·04
8 (2/5)	2½	20	—	—	154,000,000	690	<0·01	13,900,000	9·05
2 (28/9)	2½	26	—	—	470,000,000	3,370,000	0·72	5,240,000	1·08
2 (2/5)	3½	11	—	—	249,000,000	690	<0·01	174,000	0·07
32	3½* 5½	17	191,000,000	198,000,000	—	3,200	<0·01	450,000	0·23
34	3½	20	142,000,000	76,000,000	—	2,590	<0·01	806,600	1·07
10 (23/11)	4	20	—	—	19,000,000	3,000	0·02	2,000	0·01
33	4½	20	187,000,000	100,000,000	—	211,500	0·21	963,000	0·96
8 (15/2)	4½	20	—	—	289,000,000	1,400	<0·01	930,000	0·32
5 (28/9)	5	17	—	—	269,000,000	8,300	<0·01	1,810,000	0·61
29	5	23	103,700,000	98,000,000	—	43,000	0·04	15,900	0·02
5 (23/11)	5½	20	—	—	1,530,000	1,100	<0·01	2,880,000	0·19
35	6	17	65,000,000	76,000,000	—	3,120	<0·01	2,916,000	3·84
1 (3/7)	6	20	—	—	28,000,000	76	<0·01	11,200,000	40·00
6 (3/7)	6	23	—	—	59,000,000	344,000	0·58	1,000,000	1·69
11 (15/2)	6½	20	—	—	630,000,000	13,000	<0·01	589,000	0·09
28	6½	32	205,000,000	165,000,000	—	—	—	14,000,000	8·48
7 (15/2)	6½	50	—	—	45,000,000	225,800	0·5	650,000	1·44
11 (23/11)	7	20	—	—	618,000,000	13,000	<0·01	63,400,000	1·27
25	7	21½	135,000,000	134,000,000	—	11,700	<0·01	—	—
9 (28/9)	7	29	—	—	66,000,000	4,530,000	7·14	11,000	0·02
24	7	32	11,600,000	5,800,000	—	102,000	1·76	—	—
7 (2/5)	7	38	—	—	201,000,000	1,040,000	0·52	84,800,000	42·19
26	7	42	444,000,000	413,000,000	—	41,900	0·01	—	—
4 (23/11)	7½	20	—	—	52,000,000	3,000	<0·01	38,000	0·07
1 (23/11)	7½	23	—	—	24,000,000	118,400	0·49	46,400,000	100·00
6 (28/9)	7½	23	—	—	140,000,000	1,120,000	0·80	19,900,000	14·21
3 (15/2)	7½	32	—	—	529,000,000	460,000	0·09	7,700,000	1·45
2 (15/2)	7½	41	—	—	91,000,000	262,000	0·28	27,200,000	29·98
9 (3/7)	7½	41	—	—	168,000,000	3,800	<0·01	6,000	<0·01
27	7½	46	134,000,000	37,000,000	—	+1,000,000	+2·7	—	—
9 (15/2)	7½	50	—	—	197,000,000	4,452,000	2·24	414,400	0·21
6 (2/5)	8	38	—	—	168,000,000	17,500	0·01	230,000	0·14
12 (2/5)	8	50	—	—	105,000,000	—	—	4,000	<0·01
3 (28/9)	8½	39½	—	—	195,000,000	1,364,000	0·70	115,200,000	58·97
12 (23/11)	8½	44	—	—	261,000,000	1,140,000	0·43	135,800,000	51·72

* Resazurin reduction time. — No estimate made of bacteria present.

more than 50% staphylococci and nine of these had a dye-reduction time of greater than 6 hr.

In general, the high counts of staphylococci were confined to samples with reduction time in excess of 6 hr., and in these samples also, the proportion of staphylococci tended to be greater than in those samples with shorter reduction times. It would appear that as the times to reduction increase there is a tendency for samples to show increasing numbers of staphylococci, which suggests that incubation at 37·5° C. leads to selective enrichment of staphylococci.

Table 2. *The distribution of samples based on the count of staphylococci present*

Nos. of staphylococci recorded	Nos. of samples in each range		
	Initially	At time of dye reduction	At time of C.O.B. end-point
$0-10^2$	12	1	1
10^2-10^3	16	1	4
10^3-10^4	10	4	10
10^4-10^5	0	4	8
10^5-10^6	0	8	7
10^6-10^7	0	12	8
10^7-10^8	0	5	0
Totals	38	35	38

Table 3. *The distribution of samples based on the percentage of staphylococci present*

% staphylococci	Nos. of samples in each range		
	Initially	At time of dye reduction	At time of C.O.B. end-point
<1	26	9	34
1·1–5	4	5	3
5·1–10	4	3	1
10·1–15	3	2	0
15·1–20	0	2	0
20·1–30	0	2	0
30·1–40	0	1	0
40·1–50	0	1	0
>50	1	10	0
Totals	38	35	38

At the time of the end-point of the clot-on-boiling test at 22° C. there was also an increase in the numbers of the staphylococci compared with the numbers present initially. At this time, however, owing to the great increase in total colony count, thirty-four of the samples were found to contain less than 1% staphylococci, and no sample had a count greater than 10% of the total flora. Clearly staphylococci proliferate in milk held at 22° C., but it is doubtful if organisms forming such a small proportion of the total flora can play a significant part in milk spoilage.

It would seem therefore that a proportion of the flora of the majority of raw-milk samples are staphylococci. With incubation at 37·5° C., a temperature probably more favourable to staphylococci than to other types of milk bacteria, the former will proliferate at a greater rate and eventually become the dominant flora. At lower temperatures the staphylococci proliferate more slowly than other types and escape detection when the usual method of isolation of colonies from poured plates is adopted.

The rate of growth of staphylococci at 22° C. was studied in milk samples from five farms. Samples of evening milk were stored at 14° C. overnight and then transferred to the 22° C. water-bath, the time of transfer being recorded as 0 hr. The samples were plated

for total colony count at 30° C. and for staphylococcal count at 37·5° C. after 0, 6, 12, 24, 33 and 48 hr. storage at 22° C. The results are given in Table 4, and graphically in Fig. 2. The milks contained very few staphylococci initially, and, although the numbers gradually

Table 4. *The growth of staphylococci in milk during incubation at* 22° C.

Farm	Age of sample (hr.)	Total colony count at 30° C.	Count on mannitol salt agar	% of staphylococci
X	0	410	<76	<19·00
	6	500	<76	<16·00
	12	520	76	14·61
	24	1,840,000	986	0·05
	33	11,200,000	2,665,000	23·80
	48	23,600,000	226,000	0·96
X	0	950	150	15·79
	6	890	76	8·54
	12	700	530	75·71
	24	1,380,000	434,000	31·46
	33	9,200,000	2,100,000	22·83
	48	59,400,000	1,910,000	3·22
Y	0	3,440	<76	<2·21
	6	6,400	688	10·62
	12	23,200	5,900	25·43
	24	2,830,000	184,000	6·51
	33	91,000,000	187,500	0·21
	48	13,500,000	137,700	1·02
Y	0	3,800	1,200	31·58
	6	8,100	688	8·49
	12	22,800	2,500	10·95
	24	10,000,000	97,000	0·10
	33	24,700,000	97,000	0·39
	48	149,000,000	388,000	0·26
Z	0	1	<76	—
	6	10	<76	—
	12	10	<76	—
	24	1,460,000	31,700	2·17
	33	9,700,000	49,600	0·51
	48	52,000,000	29,600	0·06
Z	0	<1	<76	—
	6	<1	<76	—
	12	300	<76	<25·00
	24	15,800,000	<76	<0·01
	33	18,900,000	<76	<0·01
	48	440,000,000	<76	<0·01
A	0	1,240	<76	<6·13
	6	2,200	688	31·27
	12	20,000	1,140	5·70
	24	13,900,000	83,800	0·60
	33	30,500,000	75,600	0·25
	48	630,000,000	4,820	<0·01
B	0	30,000	980	3·27
	6	26,500	688	2·69
	12	31,200	1,200	3·88
	24	4,760,000	259,000	5·44
	33	26,000,000	278,000	1·07
	48	129,000,000	265,000	0·21

<76 = no colonies on the plate prepared from 1/10 dilution of the milk.

increased during incubation, even at 48 hr. they constituted only a small proportion of the total flora. The end-point of the clot-on-boiling test occurred in every sample between 33 and 48 hr.

From our observations, results after incubation at 37·5° C. bear no relationship to what actually occurs at 22° C., and presumably the rate of proliferation at still lower

temperatures would be even slower. The dangers to human health from staphylococci after storage at higher temperatures must not be minimized, especially when dealing with

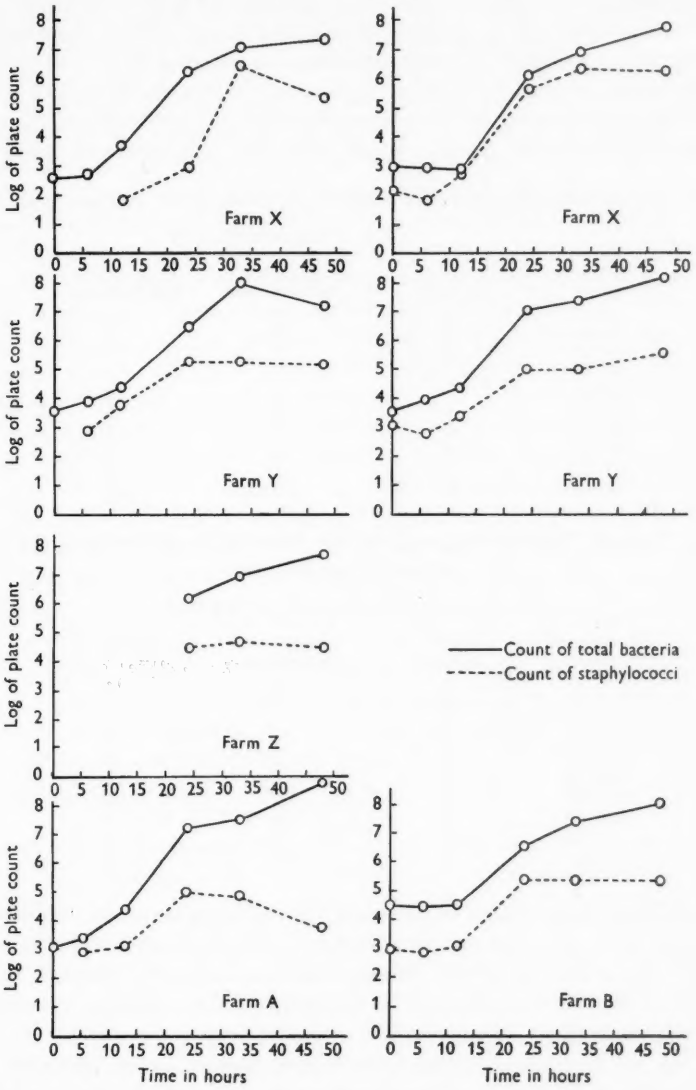


Fig. 2. Graphs showing the increase in total count and in staphylococci during incubation of milk at 22° C.

carefully produced milk from small herds in which the initial infection of staphylococci may form a high proportion of the total bacterial flora.

The incidence of the coli-aerogenes *group as indicated by plating on a differential medium*

The distribution of the numbers of the *coli-aerogenes* group is shown in Tables 1, 5 and 6.

It is now recognized that the presence in milk of members of the *coli-aerogenes* group is not necessarily an indication of faecal contamination, but that other sources of infection exist, particularly the surface of dairy equipment (Wilson (2), Mattick & Proctor (19) Sherman & Wing (20), Crossley (21). Intermammary infections are also known (Rowlands (22), Cunningham & McLeod (23)). Thus it would seem that milk produced under the most

Table 5. *The distribution of samples based on the count of the* coli-aerogenes *bacteria*

No. of *coli-aerogenes* group	Nos. in each range of count		
	Initially	At time of dye reduction	At time of C.O.B. end-point
0–10	6	0	0
10–10^2	5	0	0
10^2–10^3	10	5	0
10^3–10^4	7	9	3
10^4–10^5	4	8	4
10^5–10^6	3	6	11
10^6–10^7	0	7	7
10^7–10^8	0	2	9
10^8–10^9	0	0	2
Total	35	37	36

Table 6. *The distribution of samples based on the percentage of the* coli-aerogenes *bacteria*

No. of *coli-aerogenes* group	Nos. of samples in each range		
	Initially	At time of dye reduction	At time of C.O.B. end-point
<1	18	21	18
1·1–5	12	4	9
5·1–10	2	1	2
10·1–15	1	2	1
15·1–20	0	1	0
20·1–30	0	4	1
30·1–40	1	2	1
40·1–50	0	0	1
>50	1	2	3
Total	35	37	36

hygienic conditions may contain organisms of the *coli-aerogenes* group. Moreover, rapid multiplication of the *coli-aerogenes* group may occur in milk which is held at temperatures above 10° C. (Ayers & Clemmer (24)). Wilson (2) has emphasized this point, and maintains that unless milk has been held below 5° C. it is not possible to estimate the extent of the original contamination.

Apart from the significance of the *coli-aerogenes* bacteria as an indication of faecal contamination, it is claimed that these organisms are detrimental to the keeping quality of milk. On the other hand, Wilson (2) has shown that the actual correlation with keeping quality is poor.

Little attention has been paid to the rate of multiplication of *coli-aerogenes* bacteria in milk during storage and if, as has been claimed, these organisms play an important role in milk spoilage they would be expected to constitute an appreciable proportion of the flora at some stage.

Initially, eighteen of the thirty-five samples examined contained less than 1% *coli-aerogenes* bacteria, and twelve between 1 and 5%. It is possible that organisms which constitute only a minor proportion of the flora of the raw milk initially might become dominant during storage in the same way as has been shown to occur in pasteurized milk with other organisms (Rowlands & Provan (25), Soriano (26), Ashton (27)).

At the time of dye reduction at 37·5° C. the numbers of the *coli-aerogenes* group had increased considerably in the majority of samples, but in six no increase was recorded. The proportion of these organisms of the total flora was greater than 50% in two samples and greater than 20% in eight; on the other hand, they formed less than 1% in twenty-one samples. It would seem reasonable to assume that in the eleven samples where the *coli-aerogenes* bacteria formed more than 10% of the total flora, they had played some part in dye reduction.

At the time of the clot-on-boiling test end-point at 22° C., there was, in general, an increase in numbers over those present initially, and over those present at the time of dye reduction. Although half the samples contained over a million of the *coli-aerogenes* bacteria these organisms formed less than 1% of the total flora in eighteen samples and only in six did they form more than 15%.

Of the seventeen samples containing more than 1% of the *coli-aerogenes* group in the milk initially only two contained more than 10% and only nine more than 1% of these organisms at the time of the end-point of the clot-on-boiling test; while at the time of dye reduction seven of these seventeen samples had more than 10% and only ten more than 1% *coli-aerogenes* bacteria. It is of interest to note that *coli-aerogenes* bacteria exceeded 10% of the total flora at the time of the end-point of the clot-on-boiling test in only three of the twenty-five samples with a keeping quality of 30 hr. or less, whereas they constituted more than 30% in three of the twelve samples with a keeping quality in excess of 30 hr. Judging from the results for this series of samples the *coli-aerogenes* group rarely predominate after storage of milk at 22° C., even when they are present in considerable numbers initially or when they form an appreciable proportion of the initial flora, nor, it seems, is it safe to deduce the probable behaviour of milk on storage by a determination of the initial incidence of numbers of the *coli-aerogenes* group.

The distribution of the dominant organisms in milk initially, at the time of dye reduction at 37·5° C. *and at the end-point of the clot-on-boiling test at* 22° C.

The results of the preliminary grouping of the cultures isolated are given in Tables 7 and 8 and diagrammatically in Fig. 3.

Effect of age and treatment of the samples. The distribution of the types of organism in the milks varied according to the age and temperature of incubation of the sample. From the plates prepared initially streptococci, micrococci and achromobacteria were most frequently isolated, but members of other genera were found in most of the samples. At the time of dye reduction at 37·5° C. the main change in flora occurred in the form of an increase in the proportion of the streptococci and staphylococci and a marked decrease in the proportion of the achromobacteria and the microbacteria. At the time of the end-point of the clot-on-boiling test at 22° C. the flora was dominated by streptococci and 221 of the 265 cultures were identified as belonging to this genus. The increase in the numbers of the *coli-aerogenes* group following incubation of the samples at 22 and 37·5° C. was only slight, and at no time did these organisms constitute an appreciable proportion

Table 7. *The distribution of the cultures isolated*

No. of sample	Strepto-cocci	*Coli-aerogenes* Group	Staphylo-cocci	Other micro-cocci	Gram-positive rods	*Pseudo-monas*	*Achromo-*bacteria	*Chromo-*bacteria	Micro-bacteria	Un-classified	No growth	Total cultures
Initial milk—plates incubated at 37·5° C.												
23	4	—	—	—	1	—	1	—	11	—	1	18
25	4	—	—	20	1	—	—	—	—	1	2	28
26	—	—	16	3	—	—	—	—	—	—	—	19
27	—	—	2	5	—	—	13	—	—	—	—	20
28	3	—	8	7	—	—	6	1	—	—	2	27
29	21	—	—	1	—	—	1	—	—	—	—	23
30	1	2	—	4	1	—	7	5	—	—	3	24
31	15	4	—	—	—	—	7	—	—	—	—	26
32	3	—	—	1	—	—	8	1	8	—	—	21
33	3	—	1	10	—	—	6	—	—	—	—	20
34	17	—	—	2	—	—	8	3	—	—	—	30
35	7	—	—	2	—	—	8	—	3	—	—	20
Totals	78	6	27	55	3	0	65	10	22	1	8	275
Initial milk—plates incubated at 22° C.												
23	7	—	—	—	—	—	1	—	11	—	1	20
25	—	—	—	2	—	—	—	—	21	—	1	24
26	4	—	—	14	1	—	—	1	—	—	3	23
27	—	—	—	7	—	—	13	1	—	1	1	23
28	2	—	8	2	—	—	12	2	—	1	—	27
29	15	—	—	5	—	—	—	2	—	—	—	22
30	1	—	—	—	—	—	23	1	—	—	1	26
31	20	3	—	—	—	—	6	1	—	—	—	30
32	6	—	—	1	—	—	5	1	9	—	—	22
33	2	—	—	1	—	2	9	5	—	2	1	22
34	10	—	—	—	—	—	9	1	—	2	1	23
35	1	—	—	1	—	—	9	1	9	—	1 (discarded)	22
Totals	68	3	8	33	1	2	87	16	50	6	10	284
Time of dye reduction—plates incubated at 37·5° C.												
23	—	—	4	17	—	—	—	—	—	—	—	21
25	19	—	3	1	—	—	—	—	—	—	—	23
26	1	—	6	10	—	—	1	—	—	—	3	21
27	7	—	10	5	—	—	—	—	—	—	—	22
28	—	5	15	1	—	—	2	—	—	—	—	23
29	21	—	1	2	—	—	—	—	—	—	—	24
30	8	4	—	—	—	—	2	9	—	1	4	28
31	20	6	—	—	—	—	1	—	—	—	—	27
32	17 / 7	—	— / 11	—	—	—	5 / 3	—	—	—	—	22 / 21
33	—	—	8	18	—	—	—	—	—	—	—	26
34	21	—	—	—	—	—	—	—	—	—	2	23
35	21	—	6	—	—	—	—	—	—	—	—	27
Totals	142	15	64	54	0	0	14	9	0	1	9	308
Time of clot-on-boiling test end-point—plates incubated at 22° C.												
23	19	—	—	—	—	—	2	—	—	—	—	21
25	20	—	—	1	—	—	—	—	—	—	2	23
26	17	—	—	—	—	—	1	—	—	—	1	19
27	6	6	—	—	1	—	13	—	—	—	—	26
28	17	3	—	—	—	—	1	—	—	—	1	22
29	21	—	—	—	—	—	—	—	—	—	—	21
30	21	1	—	—	—	—	—	—	—	—	2	24
31	16	1	—	—	—	—	3	—	—	—	1	21
32	20	—	—	—	—	—	1	1	—	—	—	22
33	19	—	—	—	—	—	1	—	—	—	—	20
34	23	—	—	—	—	—	—	—	—	—	1	24
35	22	—	—	—	—	—	—	—	—	—	—	22
otals	221	11	0	1	1	0	22	1	0	0	8	265

of the total flora. The results for these twelve samples indicate that the flora in milk samples initially gives no indication of the flora which later becomes dominant during incubation at 37·5 and 22° C. in the dye-reduction and clot-on-boiling tests respectively. Even when members of the same genus were present initially and at the end-points of the tests, they were not necessarily the same species. This is most clearly seen with the streptococci. The distribution of the separate groups within this genus is given in Tables 9 and 10 and in Fig. 4. In sample 31, for example, where streptococci formed the dominant flora isolated from the initial plates and again at both test end-points marked changes occurred. Most of the cultures isolated initially were members

Table 8. *The distribution of cultures isolated*

	Strepto-cocci	*Coli-aerogenes* group	Staphy-lococci	Other micro-cocci	Gram-positive rods	*Pseudo-monas*	*Achromo-bacteria*	*Chromo-bacteria*	Micro-bacteria	Un-classified	No growth	Total cultures
					Numbers of colonies isolated							
Initial milk:												
37·5° C.	78	6	27	55	3	0	65	10	22	1	8	275
22° C.	68	3	8	33	1	2	87	16	50	6	10	284
Time of dye reduction	142	15	64	54	0	0	14	9	0	1	9	308
Time of clot-on-boiling test end-point	221	11	0	1	1	0	22	1	0	0	8	265
Totals	509	35	99	143	5	2	188	36	72	8	35	1132
					Percentage distribution of the cultures							
Initial milk:												
37·5° C.	28·32	2·19	9·85	20·01	1·09	0·00	23·62	3·64	8·00	0·36	2·92	100·00
22° C.	23·92	1·06	2·82	11·63	0·35	0·70	30·65	5·63	17·60	2·12	3·52	100·00
Time of dye reduction	46·09	4·87	20·79	17·54	0·00	0·00	4·55	2·92	0·00	0·32	2·92	100·00
Time of clot-on-boiling test end-point	83·40	4·15	0·00	0·37	0·37	0·00	8·31	0·37	0·00	0·00	3·02	99·99
Totals	45·00	3·09	8·75	12·55	0·44	0·18	16·63	3·19	6·37	0·71	3·09	100·00

of serological group E, a few belonging to group N. At the time of dye reduction at 37·5° C. the cultures were mainly group N strains (*Streptococcus lactis*), while at the end-point of the clot-on-boiling test at 22° C. group N strains were again dominant, but they were not *Str. lactis* types.

The effect of temperature is evident when the types isolated from the initial plates incubated at 22° C. and those incubated at 37·5° C. are compared. In some samples, e.g. 25, 26 and 30, the difference is very marked; the flora isolated from the two plates of other samples varied to a lesser extent. Egdell & Bird (28) observed a similar variation in the types of organism developing colonies on plates incubated at different temperatures. If, then, the organisms developing colonies on plates incubated at 37·5° C. are different from those developing colonies at 22° C., it is only to be expected that different bacteria would form the predominant flora after the milk itself had been incubated at 22° C. than when the same milk had been incubated at 37·5° C.

Observations on the different genera isolated

The streptococci. Of all the cultures isolated 45% belonged to the genus *Streptococcus.* Initially just under 30% of the cultures isolated belonged to this genus; at the time of dye reduction they formed about 46%, and at the clot-on-boiling test end-point about

83%. They were isolated from either or both of the initial plates incubated at 22 or 37·5° C., from eleven of the twelve samples, from nine at the time of dye reduction and from all twelve samples at the end-point of the clot-on-boiling test. The cultures were found to fall into serological groups, B, C, D, E and N, the heterofermentative types, and 112 formed a miscellaneous collection of unclassified strains. The distribution of the streptococci is given in Tables 9 and 10 and diagrammatically in Fig. 3.

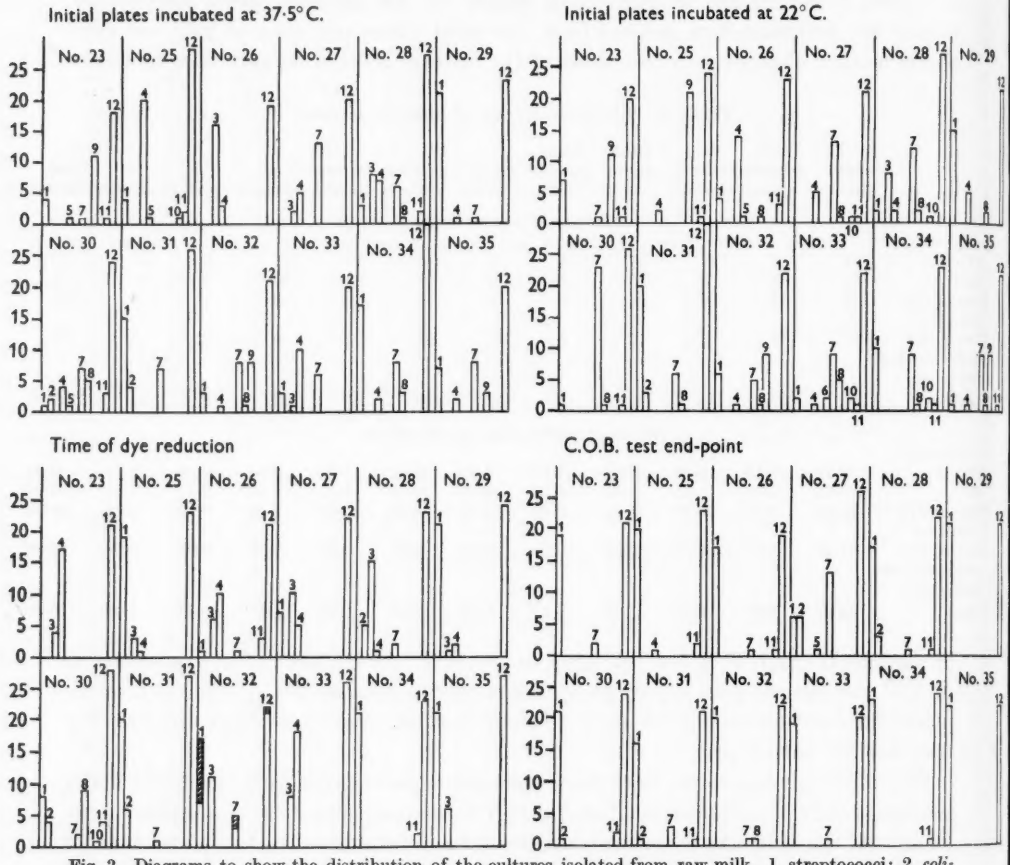


Fig. 3. Diagrams to show the distribution of the cultures isolated from raw milk. 1, streptococci; 2, *coli-aerogenes* bacteria; 3, staphylococci; 4, micrococci; 5, Gram-positive rods; 6, *Pseudomonas*; 7, achromobacteria; 8, chromobacteria; 9, microbacteria; 10, unclassified; 11, no growth; 12, total.

Sample 32, *Shaded areas* = the difference between the number of colonies isolated at the time of resazurin reduction (total area 1, 7 and 12) and the number isolated at the time of methylene blue reduction (unshaded areas 1, 3, 7 and 12).

From the public health standpoint a gradual increase of the group N or heterofermentative types during storage can have little significance, whereas proliferation of other species would be less desirable. On the other hand, rapid proliferation of group N streptococci may have an adverse effect on keeping quality and is therefore undesirable commercially. The results show that strains encouraged by incubation at 37·5° C. are different from those encouraged by incubation at 22° C., and only in sample 35 was the same strain

Table 9. *The distribution of streptococci isolated*

Sample no.	Serological group B	C	D	E	N	Hetero-fermentative streptococci	Unclassified	Total
Initial milk—plates incubated at 37·5° C.								
23	4	—	—	—	—	—	—	4
25	—	—	—	—	4	—	—	4
26	—	—	—	—	—	—	—	—
27	—	—	—	—	—	—	—	—
28	2	—	—	—	1	—	—	3
29	—	—	16	—	—	—	5 resemble group E	21
30	—	—	—	—	1	—	—	1
31	—	—	—	6	4	—	5	15
32	—	—	—	—	—	—	3 grew at 45° C.	3
33	—	—	—	—	—	—	3 resemble group E	3
34	—	—	—	—	2	—	15 most grew at 45° C.	17
35	—	—	—	—	7	—	—	7
Totals	6	0	16	6	19	0	31	78
Initial milk—plates incubated at 22° C.								
23	7	—	—	—	—	—	—	7
25	—	—	—	—	—	—	—	—
26	4	—	—	—	—	—	—	4
27	—	—	—	—	—	—	—	—
28	2	—	—	—	—	—	—	2
29	1	—	7	6	1	—	—	15
30	—	—	—	—	1	—	—	1
31	—	—	—	12	6	—	2	20
32	—	—	—	—	3	—	3 grew at 45° C.	6
33	—	—	—	—	2	—	—	2
34	—	—	—	1	1	1	7 some grew at 45° C.	10
35	—	—	—	—	1	—	—	1
Totals	14	0	7	19	15	1	12	68
Time of dye reduction—plates incubated at 37·5° C.								
23	—	—	—	—	—	—	—	—
25	—	—	—	—	11	8	—	19
26	—	1	—	—	—	—	—	1
27	—	7	—	—	—	—	—	7
28	—	—	—	—	—	—	—	—
29	—	—	11	7	3	—	—	21
30	—	—	—	—	8	—	—	8
31	—	—	3	—	14	—	3	20
32 R	—	—	—	—	—	—	17 grew at	17
32 M	—	—	—	—	—	—	7 45° C.	7
33	—	—	—	—	—	—	—	—
34	—	—	—	1	—	—	20 some grew at 45° C.	21
35	—	—	—	—	21	—	—	21
Totals	0	8	14	8	57	8	47	142
Time of clot-on-boiling test end-point—plates incubated at 22° C.								
23	3	—	6	—	10	—	—	19
25	—	—	—	—	4	16	—	20
26	—	—	—	—	17	—	—	17
27	—	6	—	—	—	—	—	6
28	—	—	—	—	17	—	—	17
29	—	—	8	3	10	—	—	21
30	—	—	—	—	21	—	—	21
31	—	—	—	—	7	—	1 + 8 lost	16
32	—	—	—	1	14	—	5 grew at 45° C.	20
33	—	—	—	—	16	2	1 resembles group E	19
34	—	—	—	—	16	—	5 resemble group N 2 grew at 45° C.	23
35	—	—	—	—	22	—	—	22
Totals	3	6	14	4	154	18	22	221

Table 10. *The distribution of streptococci isolated*

Time of isolation	Group B	Group C	Group D	Group E	Group N	Hetero-fermentative streptococci	Un-classified	Total
Initial milk:								
37·5° C.	6	0	16	6	19	0	31	78
22° C.	14	0	7	19	15	1	12	68
Time of dye reduction	0	8	14	8	57	8	47	142
Time of clot-on-boiling test end-point	3	6	14	4	154	18	22	221
Totals	23	14	51	37	245	27	112	509
Percentage distribution:								
Initial milk:								
37·5° C.	7·69	0·00	20·49	7·69	24·37	0·00	39·76	15·3
22° C.	20·59	0·00	10·30	27·94	22·07	1·46	17·64	13·4
Time of dye reduction	0·00	5·63	9·85	5·63	40·11	5·63	33·05	27·9
Time of clot-on-boiling test end-point	1·35	2·71	6·34	1·81	69·68	8·15	9·96	43·4
Totals	4·52	2·76	10·01	7·27	48·12	5·31	22·01	100·00

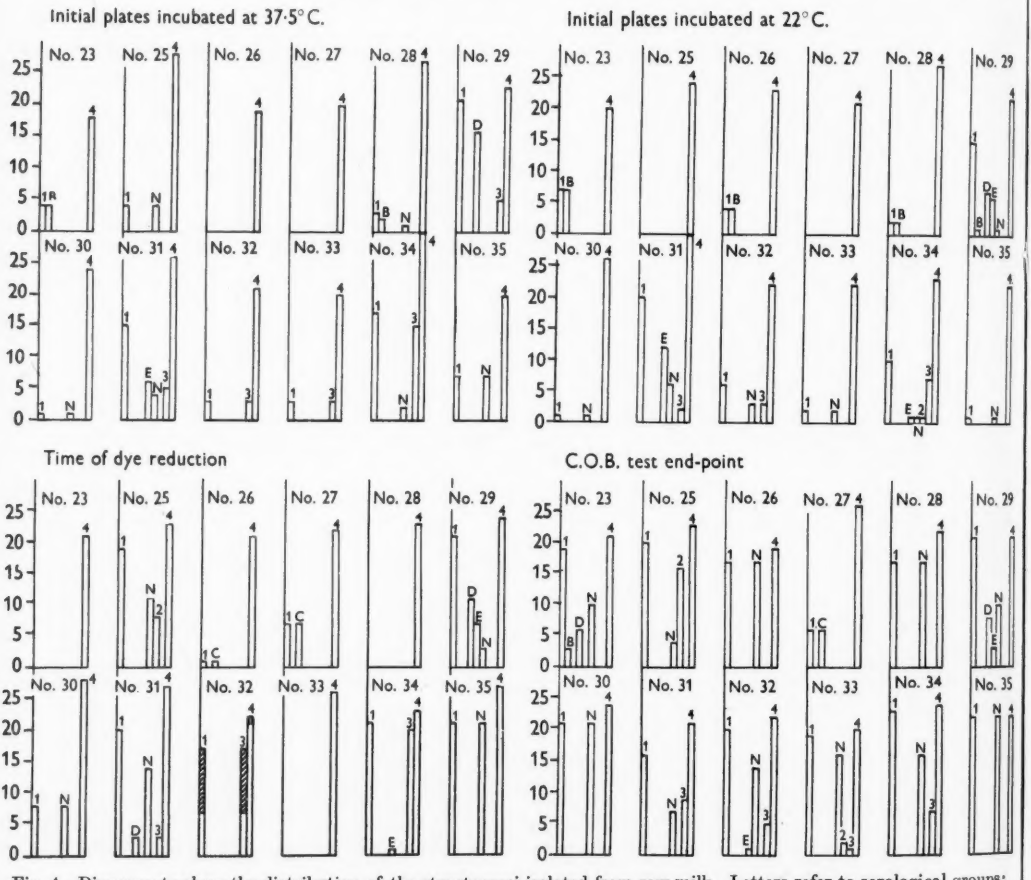


Fig. 4. Diagrams to show the distribution of the streptococci isolated from raw milk. Letters refer to serological groups: 1, total streptococci; 2, heterofermentative streptococci; 3, unclassified; 4, total cultures.
Sample 32, *Shaded areas* = the difference between the number of colonies isolated at time of methylene blue reduction (unshaded area) and resazurin reduction (total area).

found to be dominant both at the time of dye reduction and at the end-point of the clot-on-boiling test (Table 11).

Streptococci of groups B and E were found initially, but were less prevalent at the time of dye reduction and the end-point of the clot-on-boiling test. Group N strains were frequently encountered in the initial milk.

At the time of dye reduction, group N (mainly *Str. lactis*) strains formed 40·11% of the streptococci isolated and formed a major part of the flora isolated from four samples. Group D strains were dominant in one sample (29), and samples 32 and 34 both had a dominant flora of unclassified strains growing at 45° C.

At the time of clot-on-boiling group N streptococci formed 69·68% of the streptococci isolated and were the dominant flora in nine samples. Not all these cultures were strains of *Str. lactis*, however, and the dominant flora in samples 23, 30, 31 and 33

Table 11. *The cultures found to be common to both 'D' and 'C' plates**

No. of sample	Cultures common to 'D' and 'C' plates Type	No. 'D'	No. 'C'	No. of different cultures 'D'	No. of different cultures 'C'	Total cultures 'D'	Total cultures 'C'
25	*Streptococcus* (group N)	1	4	22	19	23	23
27	*Streptococcus* (group C)	7	6	15	20	22	26
28	*Bact. coli* (Type 1)	5	3	18	19	23	22
29	*Streptococcus* (group N)	3	10	11	3	24	21
	Streptococcus (group D)†	10	8				
30	*Streptococcus* (group N)	2	5	22	18	28	24
	Bact. coli (Intermediate I)	4	1				
31	*Streptococcus* (group N)	14	3	4	12	27	21
	Streptococcus (group E)	3	5				
	Bact. coli (Intermediate I)†	6	1				
32	*Streptococcus* (unclassified)†	20		2		22	
		7	5	14	17	21	22
34	*Streptococcus* (unclassified)†	19	2	4	22	23	24
35	*Streptococcus* (group N)†	21	22	6	0	27	22
	Totals	124	80				

* 'D' plates prepared at time of dye reduction.
'C' plates prepared at time of clot-on-boiling test end-point.
† Those cultures also isolated from one or both initial plates.

were found to be more closely allied to *Str. cremoris*. These cultures did not form a homogeneous group, but they had certain characters in common; they all failed to produce ammonia from arginine, they did not grow at 40° C., and they produced acid from maltose, dextrin and usually raffinose. They were not all able to grow well in skim milk, particularly on first isolation, but were encouraged by the addition of yeastrel and dextrose. These strains were not found on plates incubated at 37·5° C., but they are apparently of importance in milk spoilage.

Undoubtedly group N streptococci are more favoured by storage at 22° C. than any other group. A temperature of 37·5° C. encourages group D streptococci and other streptococci which can grow at 45° C., as well as some strains of *Str. lactis*, but it is too high to be favourable to the development of other types of group N. The group B and E types do not grow well apparently under the conditions of either test.

The keeping quality of milk may be defined as that period of time during which the milk will remain sweet under normal storage conditions at atmospheric temperature, and under these conditions raw milk can reasonably be expected to turn sour eventually with a dominant bacterial flora of group N streptococci. A test which is reflecting these

conditions can be considered to be a truer measure of the keeping quality of milk than one at 37·5° C., which encourages the development of other types of bacteria.

The micrococci. All Gram-positive, catalase-positive cocci have been included in one group. The distribution of these organisms is given in Table 12. They have been divided into staphylococci (organisms producing coagulase), orange micrococci and others. Only one culture was isolated from the plates prepared at the time of the end-point of the clot-on-boiling test. It may be inferred from these results that micrococci, including staphylococci, do not play a major role in milk spoilage at 22° C. Wilson (2) places them penultimate in his list of organisms isolated at the time of dye reduction at 17·5° C., and it seems that they are of considerably less importance in dye reduction at lower temperatures than at 37·5° C.

Table 12. *The distribution of the micrococci isolated*

	Cultures isolated from raw milk								Cultures isolated at time of dye reduction 37·5° C.			
	At 22° C.				At 37·5° C.							
Sample no.	Total cultures	Group 1	Group 2	Group 3	Total cultures	Group 1	Group 2	Group 3	Total cultures	Group 1	Group 2	Group 3
23	—	—	—	—	—	—	—	—	21	4	—	17
25	2	—	—	2	20	—	4	16	4	3	1	—
26	14	—	14	—	19	16	—	3	16	6	7	3
27	7	—	2	5	7	2	4	1	15	10	5	—
28	10	8	1	1	15	8	3	4	16	15	1	—
29	5	—	1	4	1	—	—	1	3	1	—	2
30	—	—	—	—	4	—	—	4	—	—	—	—
31	—	—	—	—	—	—	—	—	—	—	—	—
32	1	—	1	—	1	—	1	—	11	11	—	—
33	1	—	—	1	11	1	1	9	26	8	3	15
34	—	—	—	—	2	—	1	1	—	—	—	—
35	1	—	1	—	2	—	2	—	6	6	—	—
Totals	41	8	20	13	82	27	16	39	118	64	17	37
%		20·0	50·0	30·0		33·0	19·5	47·5		54·2	14·4	31·4

Group 1 = staphylococci. Group 2 = orange micrococci. Group 3 = other micrococci.

The one *Micrococcus* culture isolated at time to clot-on-boiling was from sample 25 and belonged to group 3.

Staphylococci were isolated from nine of the twelve samples at the time of dye reduction and were the dominant flora in samples 27, 28 and 32(M), all of which had a time of dye reduction in excess of 5 hr. While they were only found in a few samples initially and were comparatively unimportant, it seems that the enrichment of the staphylococci at the expense of the other organisms may occur during incubation of the samples at 37·5° C.

Sample 32 is of interest in this respect. There was a difference of 2 hr. between the time of reduction of resazurin occurring at $3\frac{1}{2}$ hr. and the time of reduction of methylene blue occurring at $5\frac{1}{2}$ hr. This difference was regarded as significant, and plates were poured from both the resazurin and methylene-blue tubes.

The colonies isolated from the plates prepared from the resazurin tube consisted of seventeen streptococci and five achromobacteria, while the colonies isolated from the plates prepared from the methylene-blue test consisted of seven streptococci, eleven staphylococci and three achromobacteria. Methylene-blue dye was observed to have a slightly toxic effect on the *Streptococcus* from this sample, a fact which probably accounted for the delay in reduction of the methylene blue as compared with resazurin, i.e. until the staphylococci had increased sufficiently to cause reduction (Garvie & Rowlands (29)). At the time of reduction of resazurin, due in the main, presumably, to the streptococci, the staphylococci had not increased sufficiently during the $3\frac{1}{2}$ hr. incubation to be isolated from the plates prepared at that time.

The achromobacteria. All non-pigmented Gram-negative rods not producing acid and gas in MacConkey broth and not producing a green fluorescent pigment have been grouped together as achromobacteria. They formed 15·6% of the total cultures handled and were isolated from ten samples initially, often in considerable numbers. They were found frequently in small numbers both at the time of dye reduction at 37·5° C. and at the end-point of the clot-on-boiling test at 22° C., but the part they played in either test end-point was, in all probability, insignificant. Had it been otherwise they would have been found to be present in greater numbers. From the observations of Wilson (2) it appears however that at 17·5° C. members of the achromobacteria play a considerable part in the reduction of methylene blue.

The microbacteria. While these organisms comprised a considerable proportion of the flora isolated initially from four samples, they were apparently unimportant in either dye reduction or milk spoilage.

The coli-aerogenes *group.* It is not possible to obtain from the twelve samples examined any information regarding the role of the *coli-aerogenes* bacteria in either dye reduction or milk spoilage. Only a very few organisms of this group were isolated, and the results obtained from the counts on violet red-bile agar were of greater value. These have been discussed already.

SUMMARY AND CONCLUSIONS

1. The present work was planned to provide an explanation of the lack of correlation for individual samples between the results of dye-reduction tests at 37·5° C. and keeping-quality tests at 22° C. A special study was made of the particular part played by the staphylococci (in thirty-eight samples) and the *coli-aerogenes* group (in thirty-seven samples) in dye reduction at 37·5° C. and in keeping quality at 22° C. A detailed study was also made of the changes in bacterial flora of twelve samples during incubation at these temperatures.

2. In milk, after overnight storage at 14° C., staphylococci were found to constitute only a small proportion of the bacterial flora, but their numbers increased during incubation at 37·5° C. (for the dye-reduction test) and at 22° C. (for the clot-on-boiling test). At the time of dye reduction staphylococci were found to constitute a major part of the bacterial flora, particularly in samples taking 5 hr. or more to reduce the dye. Although the staphylococci increased in numbers during incubation at 22° C. they constituted only a relatively small proportion of the flora at the time of the end-point of the clot-on-boiling test.

3. Initially the *coli-aerogenes* group were found to constitute only a small proportion of the total bacterial flora. Some growth usually occurred during incubation at 37·5 and 22° C. In some samples members of the *coli-aerogenes* group became dominant by the time of reduction of the dye at 37·5° C. and also at the time of the end-point of the clot-on-boiling test at 22° C. in others. However, there appeared to be no relationship between the numbers or proportion present initially and the numbers or proportion present at either time of dye reduction or at the end-point of the clot-on-boiling test. It seems therefore that the extent of initial contamination with these organisms bears no relation either to the time to dye reduction or to the time to reach the end-point of keeping quality.

4. A study of the dominant bacterial flora from milk initially, at the time of dye reduction, and at the time of the clot-on-boiling test end-point, has revealed considerable differences at the different times. Achromobacteria and microbacteria were found in considerable numbers initially but apparently played little part in either test.

Incubation at 37·5° C. encouraged the staphylococci, micrococci and streptococci of various groups, notably groups N and D, and also some unclassified strains growing at 45° C. Incubation at 22° C. encouraged mainly group N streptococci. Further, in some samples where streptococci became dominant at both end-points different species were found. Considerable differences were observed with cultures isolated from plates from the same sample initially after incubation at 22 and 37·5° C.

5. The differences between the types predominating in milk samples after incubation at 37·5 and 22° C. probably account for the discrepancies between the results of dye tests at the higher temperature and keeping quality at the lower temperature. It would seem that incubation at 37·5° C. favours the rapid development of types which proliferate only slowly at 22° C., and may therefore be expected to play a relatively insignificant part in the spoilage of milk under normal conditions of household storage.

When incubation at 37·5° C. is sufficiently prolonged staphylococci tend to predominate over other types of organisms. However, it does not follow that this affords an indication of possible danger to human health, for with incubation at lower temperatures, as occurs commercially, their rate of proliferation may be relatively slow.

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472. STUDIES IN THE BACTERIOLOGY OF MILK

III. THE CORYNEBACTERIA OF MILK

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In the first two papers of this series(1, 2) an account was given of the streptococci, staphylococci and micrococci detected among the dominant micro-organisms in samples of milk. Isolations were made from quantitative plates of a glucose (0·25%), milk (5%), peptone (1%) meat infusion agar which was expected to be relatively non-selective for the commoner milk bacteria. The plates were incubated for 5–6 days at 30° C., which likewise was designed to introduce no selective factor. Transfers were made from a representative sample of colonies on plates of a suitable dilution. With the object of securing a wide range of milks, the samples examined were taken from single cows, single herds and bulked supplies. Thirty-eight of the samples were examined in the raw state and sixty-nine immediately after pasteurization in the laboratory. The organisms that multiplied in twenty-eight of the pasteurized samples were also examined. Corynebacteria were numerically important in a considerable proportion of the samples. Data on their occurrence in raw and pasteurized milk has been presented in preliminary reports(3, 4); the present paper discusses their properties and classification.

In the milk samples examined, two main types of corynebacteria were recognized: (1) a series of heat-sensitive diphtheroid organisms, which appear to be derived principally from the animal body, and (2) thermoduric saprophytes, which are introduced into milk from dairy equipment. The standard of heat resistance used in this separation is survival in milk at 63° C. for 30 min. Higher temperatures would afford the same differentiation; lower ones have not been tried.

THE HEAT-SENSITIVE DIPHTHEROID ORGANISMS

Bacterium lipolyticum Evans was found in several of the uncontaminated single-cow samples, but in milk produced by ordinary methods it was detected in only two of the purest single-herd supplies. Since this organism is biochemically inactive in milk, and since the colonies which it might occasionally produce on milk-count agars are so small that they would easily escape detection, its occurrence in milk appears to be of little practical interest. Although *Bergey's Manual*(5) indicates that this organism may be identical with *Corynebacterium bovis*, the two types appear to be appreciably different.

The remaining heat-sensitive diphtheroids were isolated partly from samples secured with aseptic precautions from single cows and partly from ordinary raw milk. This distribution indicates that, unlike *Bact. lipolyticum*, they were not derived solely from the ducts of the udder. Several are similar to if not identical with organisms which occur on the skin and in laboratory air. The cells of the majority are short and frequently coccal, and confusion with staphylococci is not impossible, especially as the two forms simulate

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each other in colonial character. The majority did not form pigment, showed no action on lactose, casein or fat, and produced an alkaline reaction or no change in litmus milk. A minority differed in one or more of these characters, and certain other variations were noted. Since the organisms did not appear to be among the more important milk bacteria they were not studied in detail.

THE THERMODURIC CORYNEBACTERIA

Organisms of this type were never detected in samples taken directly from the udder but, with the exception of a few of the purest herd supplies, they were obtained from every specimen of market milk examined. A total of 2502 cultures were isolated as follows:

From raw market milk	80
From milk pasteurized in the laboratory:	
for 30 min. at 63° C.	1969
for 5, 10 or 15 min. at 80° C.	80
From dairy equipment (swabs rinsed in sterile milk which was heated 30 min. at 63° C. or 15 min. at 75° C. before plating)	373

All the cultures enumerated above were transferred to litmus milk and, later, to agar slopes and examined for morphology and catalase production. The majority, including representatives from each source, were characterized in greater detail. The organisms were found to have the following characters: cells small, 0·4–0·5 μ thick (after staining) and varying from almost coccal to about 2 μ long; non-motile; without spores; Gram-positive; growth on nutrient agar (30° C.) slow in appearing and thin; better growth if the agar contains 1–5% milk; catalase and diastase produced; acid formed from glucose and lactose; no hydrolysis of fat.

The cultures varied in pigment production (from none to a pale lemon-yellow colour), in liquefaction of gelatin (which never started before the ninth day at 22° C.), in action on litmus milk (acid, acid clot or no change), and in the reduction of nitrate to nitrite. A small minority differed from the others in bringing about a slight digestion of casein in milk agar or casein agar. In raffinose fermentation there was also a minority group which formed acid. A few strains produced on milk agar a rough adherent growth which resisted disintegration.

The characters of the cultures, especially the remarkable resistance to heat demonstrated by the methods used in isolating some of them, place the organisms in *Microbacterium* Orla-Jensen (6). Many of the cultures were clearly identifiable with *M. lacticum*, but the position of others was uncertain. *Microbacterium* has given rise to considerable confusion, partly because of inadequate original characterization, and partly because it contained elements which should not have been grouped together. Robertson (7), Wittern (8), Speck (9) Mosimann (10), McKenzie, Morrison & Lambert (11) and Doetsch & Pelczar (12) have used the name and have differentiated the constituent species in a variety of ways. *M. lacticum*, *M. flavum* and *M. liquefaciens* have been reported as occurring in pasteurized milk by Robertson (7), Abd-el-Malek (4), Speck (9), McKenzie *et al.* (11), Thomas, Jones-Evans, Jones & Thomas (13) and Doetsch & Pelczar (12).

In view of the frequency with which the organisms of this group were detected in our work, it seemed desirable to obtain additional information on their properties and if possible to place their identification on a firmer basis. A collection of 154 cultures was

built up and, after ensuring purity by plating, they were studied more intensively. The cultures were selected so as to include any variations that had been detected, and also the different sources and methods of isolation.

Characters of representative strains (154 *cultures*)

In addition to the features already mentioned the cultures were found to be uniform in several properties which assist identification. They are unable to grow in broth + 10% NaCl or in media in which an ammonium salt is the only source of nitrogen, and they show no evidence of utilizing paraffin as a source of carbon. Aesculin is split somewhat slowly. Every culture produces diastase on starch agar, but acid formation in starch broth was not always obtained. Neither natural fats nor tributyrin are atacked, and ammonia is not formed from peptone or arginine. The reduction of nitrate, when it occurs, does not proceed beyond the nitrite stage. The maximum temperature for growth is lower than 37° C. The organisms appear to be strict aerobes; no naked-eye evidence of growth was detected when inoculated glucose agar was incubated anaerobically in a McIntosh & Fildes jar containing 5% CO_2. This observation is at variance with previous reports of the anaerobic growth of such organisms by Wittern (8), Speck (9) and Doetsch & Pelczar (12). Shake and stab cultures did not give a clear indication of the oxygen relationship, presumably because growth is slow.

Gelatin liquefaction, casein digestion and pigment formation, in which variations had been noted, proved to be indefinite characters. Of the 154 cultures, 99 started to liquefy gelatin (at 22° C.) after various periods ranging from 9 to 70 days. The tubes, which were stoppered, were discarded after 70 days. Four cultures produced a weak clearing on casein agar; of these only one was a gelatin liquefier. The yellowish pigmentation of colonies varied with the medium, but not always in proportion to the luxuriance of the growth. On peptone meat extract agar both growth and pigmentation were suboptimal. The addition to it of 0·25% yeast extract (yeastrel) resulted in a medium which was the best of those tested, both for promoting growth and for intensifying the greenish yellow colour. The addition of 20% milk gave a smaller improvement. Glucose reduced both growth and pigmentation, presumably because it permits acidification. Potato showed colours fairly well, and on this medium the growth of two cultures was at first yellowish and later brown. Among the 154 cultures, only two failed to show some evidence of pigment formation on the better media.

Table 1 shows the distribution in the 154 representative cultures of certain characters that have been used for differentiating the species of *Microbacterium*. The table shows only one distinct correlation; the twenty-three raffinose fermenters differ from the other cultures in being destroyed when heated in milk for 30 min. at 75° C. A few of them survived for 15 min. at 75° C. but in greatly reduced numbers. The raffinose fermenters also produce on artificial media a scantier growth than the others, and they are among the least active acid formers in litmus milk. Nevertheless, on the evidence obtained, they do not constitute a very distinctive group.

Differentiation

None of the strains has the morphology of *Nocardia* (*Proactinomyces*) or the ability to grow with inorganic nitrogen or with paraffin as a carbon source which, according to Wittern (8) and Jensen (14), are characters of *M. mesentericum*. The few cultures that

formed an adherent finely wrinkled growth, suggestive of Orla-Jensen's (6) original description of that species, showed no other distinctive feature, and it was possible to separate from some of them variants which produced a smooth friable growth like that of other strains.

The original description of *M. flavum* Orla-Jensen (6) was based on two strains which have been re-examined and further characterized by Wittern (8), H. L. Jensen (15), A. D. Orla-Jensen (16) and Doetsch & Pelczar (12). Other strains have been isolated and described by Wittern (8), Speck (9), Mosimann (10) and McKenzie *et al.* (11). This species is regarded by H. L. Jensen (15) as an intermediate between *Mycobacterium* and *Corynebacterium*. While the limits of its characters are not entirely clear, it appears to differ from all the cultures described in the present paper in the larger size of its cell, in its growth on

Table 1. *Distribution of characters in representative strains*

		No. of cultures	Gelatin liquefaction +	Gelatin liquefaction −	Casein digestion +	Casein digestion −	Nitrite formation +	Nitrite formation −	Litmus milk acid +	Litmus milk acid −	Acid from maltose +	Acid from maltose −	Acid from raffinose +	Acid from raffinose −
Total		154												
Survival at 75° C. 30 min.	+	131	89	42	4	127	65	66	124	7	127	4	0	131
	−	23	10	13	0	23	9	14	18	5	23	0	23	0
Gelatin liquefaction	+	99	—	—	1	98	63	36	91	8	96	3	10	89
	−	55	—	—	3	52	11	44	51	4	54	1	13	42
Casein digestion	+	4	—	—	—	—	2	2	3	1	4	0	0	4
	−	150	—	—	—	—	72	78	139	11	146	4	23	127
Nitrite formation	+	74	—	—	—	—	—	—	68	6	73	1	9	65
	−	80	—	—	—	—	—	—	74	6	77	3	14	66
Litmus milk acid	+	142	—	—	—	—	—	—	—	—	139	3	18	124
	−	12	—	—	—	—	—	—	—	—	11	1	5	7
Acid from maltose	+	150	—	—	—	—	—	—	—	—	—	—	23	127
	−	4	—	—	—	—	—	—	—	—	—	—	0	4
Acid from raffinose	+	23	—	—	—	—	—	—	—	—	—	—	—	—
	−	131	—	—	—	—	—	—	—	—	—	—	—	—

artificial media, which is more profuse and of a deeper yellow colour, in its failure to attack aesculin, and in its ability to grow at 37° C. and to grow in the presence of 10% NaCl. Since *Microbacterium flavum* was not detected in the numerous isolations which were made, it cannot be regarded as a common contaminant of milk.

Evidently all our cultures should be classified with those microbacteria that H. L. Jensen (15) transferred to *Corynebacterium*. For reasons to be stated presently his nomenclature will be used here. There is no difficulty in identifying many of the cultures with *C. lacticum*. The question arose whether the gelatin- or casein-digesting strains should be regarded as *C. liquefaciens*. They were so named in a preliminary communication on this work (Abd-el-Malek (4)), but, after more intensive study, we have reached the conclusion that none of the cultures isolated from milk belongs to that species. Among these organisms gelatin liquefaction and casein digestion are slow and unrelated actions, and clotted milk cultures never show a separation of whey.

From poorly cleansed milk churns we have isolated organisms that appear to be typical *C. liquefaciens*. They withstand heating in milk for 30 min. at 63° C., but differ from *C. lacticum* in their more profuse growth on artificial media, in liquefying gelatin within seven days and in failing to produce diastase. Most differ also in clearing milk agar fairly rapidly, and in showing in litmus milk a weak acidity with clotting and whey separation. From glucose and lactose some form very little acid. Considering the varia-

tions found by Topping (17) in soil strains of the *C. liquefaciens* group these cultures are remarkably uniform. Since they were found in churns they are likely to occur also in milk, but on the evidence at present available *C. liquefaciens* is a relatively uncommon organism in milk.

Speck (9) and Doetsch & Pelczar (12) have isolated from milk a few strains of raffinose fermenters which they allot to *Microbacterium*. These organisms are described as relatively large coccobacilli which are variable in character of colony, in action on maltose, starch and aesculin, and in ability to grow in the presence of 10% NaCl. They, like *M. flavum*, were found by Doetsch & Pelczar (18) to differ from *M. lacticum* in nutritive requirements. The organisms appear to be unlike all those isolated from milk by us.

The characters of Corynebacterium lacticum

The foregoing discussion strongly suggests that the whole series of 154 cultures is essentially homogeneous. Many appear to be identical with *Corynebacterium lacticum*. Consequently, the characterization of that species might well include the several deviations noted above, more especially those shown in Table 1. These include the possibility of raffinose fermentation, the occasional inability to ferment maltose, the variability in the reduction of nitrate to nitrite, the uncommon slow digestion of casein, the slow liquefaction of gelatin by many strains, and the variation in heat resistance. Properties of the organisms that have not been found to vary are diastatic action on starch agar and a weak hydrolysis of aesculin.

Applying the evidence that has been presented, it is suggested that in work with dairy products an organism could be identified with reasonable certainty as *Corynebacterium lacticum* by the use of three media as follows:

(1) Agar slope (30° C.): growth relatively scanty, unpigmented to faintly yellow and giving a catalase reaction; containing short, irregular, non-sporing, Gram-positive rods about 0·5μ thick.

(2) Gelatin (22° C.): no liquefaction within seven days.

(3) Lactose (30° C.): acid.

The most likely source of error in this scheme would be confusion with the slow-growing type of thermoduric *Micrococcus* described previously by Abd-el-Malek & Gibson (2) as group IIIB.

THE STATUS OF THE GENUS *MICROBACTERIUM*

Orla-Jensen (6) assembled under the name *Microbacterium* a somewhat heterogeneous collection of Gram-positive rods which are neither lactobacilli nor propionic acid bacteria nor spore-formers. He appears to have been well aware of the taxonomic limitations of the proposed group, for at a later date he (19) refers to *Microbacterium* 'as merely a provisional collective name'. H. L. Jensen (14, 15) has done much to clarify the systematic position of the four types to which specific epithets had been given. His proposals, if accepted, undoubtedly require the suppression of *Microbacterium*. *M. lacticum*, which has been adopted by *Bergey's Manual* (5) as the type species of that genus, is transferred by H. L. Jensen (15) to *Corynebacterium*. As a contribution to this problem in nomenclature a brief statement follows.

The organisms that we have identified as *C. lacticum* possess the microscopical characters of corynebacteria, notably the irregularity of cell form, the absence of motility, the granular staining, the retention of Gram's stain, and the method of fission which

results in the cells forming angles with one another. Their physiological properties, such as their aerobic nature, catalase production and weak acid formation from sugars, are likewise typical of corynebacteria. Speck (9) has objected to their inclusion in *Corynebacterium* on the grounds that they form lactic acid as the main product of their action on sugars. The basis of this contention remains to be established, since little is known concerning the fermentation products of the corynebacteria. In utilizing glucose *C. diphtheriae* is reported by Fujita & Kodama (20), Tasman & Brandwijk (21) and Friedemann (22) to form lactic acid along with other substances, the products apparently varying with the strain and the degree of aeration. Jensen & Swaby (23) detected formic, acetic and lactic acids among the products of the decomposition of cellulose by unnamed corynebacteria. Whatever the outcome of work on the metabolism of other species of *Corynebacterium*, it may be noted that various microbial genera, as they are defined at present, accommodate species capable of producing lactic acid as the main dissimilation product of sugars. This applies to *Bacillus* (Andersen & Werkman (24)), *Micrococcus* (Cunningham & Smith (25)), *Streptomyces* (Woodruff & Foster (26)) and *Rhizopus* (Waksman & Hutchings (27)). Suggestions that *Lactobacillus* is the genus most closely related to *Microbacterium* do not receive support from the biochemical work of Hansen (28).

The possibility remains that *Microbacterium* could be separated from *Corynebacterium* on the basis of heat resistance. Orla-Jensen (6) showed that some strains of *Microbacterium* possess the property, remarkable in bacteria that do not form spores, of tolerating a temperature of 80–85° C. for 15 min. McKenzie *et al.* (11) note instances of survival in milk at 90° C. for 10 min. In sharp contrast, *Corynebacterium diphtheriae*, the type species of *Corynebacterium*, is known to be quickly destroyed at 58–60° C. Here, again, it is impossible to assess the situation, since the resistance of other corynebacteria is largely unknown. The difficulties in using heat resistance as a differential criterion are considerable. This has been demonstrated in *Streptococcus*, where the differences between species are relatively large (Abd-el-Malek & Gibson (1)), and also in the *Staphylococcus-Micrococcus* group (Abd-el-Malek & Gibson (2)).

In view of all these uncertainties, and also of the known features of the organisms, we suggest that the best immediate solution of the problem is the acceptance of Jensen's (15) proposal to allot to *Corynebacterium* those species of *Microbacterium* that have not been transferred to *Nocardia* or *Mycobacterium*.

SUMMARY

The corynebacteria that were isolated by non-selective methods from raw and pasteurized milk are of two main types: (1) heat-sensitive diphtheroid organisms which appear to be derived principally from the animal body, and (2) thermoduric saprophytes originating from dairy equipment. The characters of group (2), which are discussed in detail, indicate that all the cultures examined belong to a single species, *Corynebacterium lacticum*. Reasons are given for classifying that species in *Corynebacterium* and for suppressing the genus *Microbacterium*.

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473. THE SEROLOGICAL CLASSIFICATION OF *STREPTOCOCCUS CREMORIS*

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In 1943 Mattick & Shattock (8) described the preparation of a specific precipitating serum for *Streptococcus lactis*, and established a new serological group of ('lactic') streptococci to which the letter N was assigned. Shattock & Mattick (18) also showed that hydrochloric acid extracts of *Str. cremoris* precipitated with their 'lactis' serum and thus that *Str. cremoris* belonged to the same group. More recently, Swartling (19) has grouped *Str. lactis*, *Str. diacetilactis* and *Str. cremoris* by similar means, and his observations have been confirmed by Briggs (2).

Unequivocal identification and classification of *Str. cremoris* is desirable because of its importance in milk and dairy products; the previous work, though strongly suggestive, does not afford conclusive proof of the group classification of *Str. cremoris*, since this organism has not yet yielded a specific precipitating group serum (18, 19). The object of this work was to produce such a serum, on which final confirmation of the serological identity of *Str. cremoris* with group N must depend.

MATERIALS AND METHODS

Bacterial cultures

The majority of strains used in this investigation were obtained from the N.I.R.D. collection of freeze-dried cultures; these included *Str. lactis* OJ, Rice and M3, and *Str. cremoris* HP, RW, J and 95/2. This collection also provided strains of groups other than N for specificity tests.

Dr A. Hirsch kindly gave us thirty-one fresh cultures of lactic streptococci isolated from raw milk in France, of which we used *Str. lactis* H_{14} and *Str. cremoris* H_{19} and H_{22}, and a freeze-dried culture of *Str. cremoris* 651/10. Miss M. Baron sent us fifteen starter cultures and Mr W. A. Hoy helped us to obtain cultures from the raw milk of the N.I.R.D. and other farms. Not all of these cultures were used in this investigation, some strains merely being retained for subsequent typing.

Str. diacetilactis no. 2 was received from Prof. Eug. Pijanowski and Dr J. Supińska, Poland, and the lactic streptococcus 736 from Prof. M. Seelemann.

Biochemical and physiological tests

The biochemical and physiological tests used for the differentiation of *Str. lactis* and *Str. cremoris* were essentially those described in detail by Swartling (19). We have attached particular significance to those tests which on repeated use have been found to give the most clearly differential and consistently reproducible results, namely, maltose fermentation, growth at 40° C. and the hydrolysis of arginine (11). Nichols & Hoyle (10) also consider that, along with their reaction in litmus milk, *Str. lactis* and *Str. cremoris* can be differentiated by the latter two tests.

Serological methods

Preparation of antisera

Antisera were prepared in rabbits against living, disintegrated, heat-killed and serum-treated streptococci; in each instance the suspension was made up to an opacity equivalent to that of Brown's tube no. 10, and 1·0 ml. was injected intravenously at 3–4-day intervals.

Suspensions of living bacteria were prepared by centrifuging the cells from 24 hr. cultures in dextrose Lemco broth (DLB)(19) at 30° C. and resuspending them in 0·85% saline. The organisms in similar suspensions were partially disintegrated by shaking for 1 hr. with carborundum (grade 100, 0·5 g. per 10 ml. suspension) in a magnetic shaker (9, 17). The heat-treated suspensions were prepared by heating saline suspensions of streptococci at (*a*) 56° C. and (*b*) 60° C. for 1 hr. The suspensions described above were freshly prepared for each inoculation.

In order to inactivate certain non-specific antigens in the *Str. cremoris* cells, these were removed by 'absorption' with specific antisera for streptococcal groups K and L, before the immunizing suspensions were made up(1). This procedure should be differentiated from the usual process of absorption of serum antibodies, as referred to below; the K and L group sera used were those in routine production at this Institute(12).

In these 'absorptions' excess of serum was ensured by mixing 6 parts by volume of serum to 1 part of packed bacterial cells; the mixture was incubated at 40° C. for 2 hr. and was then stored in the refrigerator (4° C.) overnight; the cells were removed, washed in saline and resuspended in 20 ml. of 0·25% formol-saline, and sufficient suspension for the whole course of inoculations was thus produced at one time. The sera used for 'absorption' of the cell components were tested after completion of the process to confirm that the antibodies had been wholly or partly removed; it is presumed, of course, that during 'absorption' antigen-antibody combination occurred, with consequent inactivation of the antigen concerned.

Preparation of extracts

Hydrochloric acid extracts of the streptococci were prepared by Lancefield's(7) method, and formamide extracts according to Fuller's(3) technique.

Precipitin tests

Precipitin ring tests with sera and extracts were made as described by Shattock(17). The tests were read repeatedly between 1 and 10 min. and the negatives again after 15 and 30 min.

Absorptions

Absorption of serum antibodies (in contradistinction to the process of removal of cellular antigens described above) was carried out using Shattock's(17) method, save that the organisms were grown in DLB for 24 hr. and the absorption was made at 40° C. for 2 hr. before the suspension was refrigerated (4° C.) overnight.

RESULTS

Biochemical and physiological reactions

The biochemical and physiological properties of most of the strains of lactic streptococci used in this investigation have already been given in detail by Swartling(19), and, since the results of our tests agreed with his, it is unnecessary to repeat them here. However,

Table 1 shows the results of the tests, not serological, which we, with Nichols & Hoyle (10), consider to be the most useful in differentiation.

On the basis of morphology, salt tolerance and salicin fermentation only, Seelemann & Carstens (14) believe the lactic streptococcus 736 to be a strain of *Str. cremoris*. But when further differential tests were used (Table 1) it appeared that this organism is, in fact, *Str. lactis*. Sharpe (16) records similar observations on 736, and serological evidence in support of this opinion is presented later.

Table 1. *Differential biochemical and physiological reactions of lactic streptococci*

Strain	Growth at 40° C. in		Fermentation of maltose	Hydrolysis of arginine
	D.L.B.	Litmus milk		
Str. lactis OJ	+	+	+	+
Str. lactis Rice	+	+	+	+
Str. lactis M3	+	+	+	+
Str. lactis H_{14}	+	+	+	+
Str. cremoris HP	−	−	−	−
Str. cremoris RW	−	−	−	−
Str. cremoris J	−	−	−	−
Str. cremoris 95/2	−	−	−	−
Str. cremoris 651/10	−	−	−	−
Str. cremoris H_{19}	−	−	−	−
Str. cremoris H_{22}	−	−	−	−
Str. diacetilactis no. 2	+	+	+	+
Strain 736	+	+	+	+

Serological results

Hydrochloric acid extracts of all the strains of lactic streptococci precipitated with group N sera, but not with sera from any of the other streptococcal groups.

Streptococcus lactis *group sera*

Group N precipitating sera were prepared in rabbits against a number of strains of *Str. lactis* after 8–10 inoculations. The sera were shown to possess group and not merely type antibodies by their precipitation with extracts of heterologous group N strains comprising *Str. lactis*, *Str. cremoris* and *Str. diacetilactis*. Their specificity was established in precipitin tests against HCl extracts of representative strains of each of the Lancefield groups, and against a strain of *Staphylococcus* rich in the non-specific nucleoprotein fraction (Lancefield (6)).

The immunizing suspensions consisted of living, disintegrated or heated (56 or 60° C. for 1 hr.) *Str. lactis* cells, and not all the sera obtained by these means were group-specific; they were rendered so where necessary by absorption with strains of streptococci of the Lancefield groups with which they showed cross-reactions, or, sometimes, with the *Staphylococcus* strain.

The most rapidly produced, most potent and most consistently specific sera were those obtained with organisms heated at 56° C. for 1 hr.; these sera were used in the absorption tests described later.

Streptococcus cremoris *group sera*

Despite the use of a number of different freshly isolated and freeze-dried strains of *Str. cremoris* in living, disintegrated and heat-treated forms, sera were obtained which were invariably non-specific. The sera were tested for specificity and for potency as for the *Str. lactis* sera. In some instances cross-reactions were obtained with all or nearly all

the Lancefield groups, but most commonly the non-specificity was confined to groups K and L(19). In either case absorption with a streptococcus of group K or L removed cross-reacting antibodies (Table 2), while only slightly impairing the group N reactions.

It was clear that, in order to produce a specific group N serum for *Str. cremoris*, it was necessary, before the immunization process was begun, to eliminate or inactivate the

Table 2. *Absorption of non-specific* Streptococcus cremoris *sera with streptococci of groups K and L*

		Sera					
		Str. cremoris HP (vaccine: disintegrated cells)			*Str. cremoris* HP (vaccine: heated 60° C. × 1 hr.)		
			Absorbed with			Absorbed with	
Lancefield group	Representative organism.. HCl extract	Un-absorbed	Strain Sissons group K	Strain SHC 282 group L	Un-absorbed	Strain Sissons group K	Strain SHC 282 group L
A	Strain S43/100/8	+	−	−	−	−	−
B	Strain N.C.T.C. 8181	+	−	−	−	−	−
C	Strain C4	+	−	−	−	−	−
D	Strain 98 D	−	−	−	−	−	−
E	Strain C24 B	−	−	−	−	−	−
F	Strain H60 R	+	−	−	−	−	−
G	Strain M254	+	−	−	−	−	−
H	Strain Challis	−	−	−	−	−	−
K	Strain Sissons	+	−	±	+	−	−
L	Strain SHC282	+	−	−	+	−	−
M	Strain SHC241	−	−	−	−	−	−
	'Staphylococcus'	+	−	−	−	−	−
N	*Str. lactis* OJ	+	+	+	+	+	+
	Str. lactis Rice	+	+	+	+	±	+
	Str. lactis M3	+	+	+	+	+	+
	Str. cremoris HP	+	+	+	+	+	+
	Str. cremoris RW	+	+	+	+	+	+
	Str. cremoris J	+	+	+	+	+	+

Table 3. *'Absorption' of non-specific* Streptococcus cremoris *antigens with antisera of groups K and L: antibody content of unabsorbed and absorbed sera*

		Sera						
		Strain Sissons: group K					Strain SHC176: group L	
			Absorbed with *Str. cremoris*					Absorbed with
Lancefield group	Representative organism. HCl extract	Unabsorbed	RW	J	95/2	H_{19}	Unabsorbed	*Str. cremoris* H_{22}
K	Strain Sissons	+ + +	−	+ +	+ +	+	.	.
L	Strain SHC282	.	.	.	.	.	+ + +	+
N	*Str. cremoris* H_{19}	.	.	.	.	.	(+)	−
	Str. cremoris H_{22}	.	.	.	.	.	(+)	−

The number of + signs is an arbitrary indication of the intensity and rapidity of development of the reactions.

antigen in the 'cremoris' cells stimulating the production of antibodies shared with groups K and L. Since the absorption of non-specific sera with K and L streptococci did not seriously impair their group N reactions, the simple method of eliminating the antigen in question was to inactivate it by means of antigen-antibody combination. Accordingly, strains of *Str. cremoris* were 'absorbed' with sera of groups K and L as described earlier, tests of the sera after absorption showing elimination of, or more commonly a reduction in, antibody content (Table 3).

The precise nature of the antigen which *Str. cremoris* possesses in common with the streptococci of groups K and L has not been determined. However, the weak precipitin reactions between the extracts of *Str. cremoris* H_{19} and H_{22} and the unabsorbed group L serum SHC 176 were of a disseminated, 'fluffy' nature, suggesting a type antigen precipitation.

With suspensions of 'absorbed' *Str. cremoris* potent group-specific sera were obtained without difficulty after 10–14 inoculations. In some instances the organisms were disintegrated in the magnetic shaker, or heated at 56 or 60° C. either before or after

Table 4. *Absorption tests of* Streptococcus lactis *and* Streptococcus cremoris *group sera.*

		Sera							
		Str. lactis OJ (vaccine: disintegrated cells)		*Str. lactis* H_{14} (vaccine: heated 56° C. × 1 hr.)		*Str. cremoris* H_{19} (vaccine: 'absorbed' with group K serum, strain Sissons)		*Str. cremoris* H_{22} (vaccine: 'absorbed' with group L serum, strain SHC 176)	
Lancefield group	Representative organism. HCl extract	Unabsorbed	Absorbed with *Str. cremoris* HP	Unabsorbed	Absorbed with *Str. cremoris* HP	Unabsorbed	Absorbed with *Str. lactis* OJ	Unabsorbed	Absorbed with *Str. lactis* OJ
A	Strain S43/100/8	–	–	–	–	–	–	–	–
B	Strain N.C.T.C. 8181	–	–	–	–	–	–	–	–
C	Strain C4	–	–	–	–	–	–	–	–
D	Strain 98D	–	–	–	–	–	–	–	–
E	Strain C24B	–	–	–	–	–	–	–	–
F	Strain H60R	–	–	–	–	–	–	–	–
G	Strain M254	–	–	–	–	–	–	–	–
H	Strain Challis	–	–	–	–	–	–	–	–
K	Strain Sissons	–	–	–	–	–	–	–	–
L	Strain SHC282	–	–	–	–	–	–	–	–
M	Strain SHC241	–	–	–	–	–	–	–	–
	'Staphylococcus'	–	–	–	–	–	–	–	–
N	*Str. lactis* OJ	+	–	+	–	+	–	+	–
	Str. lactis Rice	+	–	+	–	+	–	+	–
	Str. lactis M3	+	–	+	–	+	–	+	–
	Str. lactis H_{14}	+	–	+	±	+	–	+	–
	Str. cremoris HP	+	–	+	–	+	–	+	–
	Str. cremoris RW	+	–	+	–	+	–	+	–
	Str. cremoris J	+	–	+	–	+	–	+	–
	Str. cremoris 95/2	+	–	+	–	+	–	+	–
	Str. cremoris 651/10	+	–	+	–	+	–	+	–
	Str. cremoris H_{19}	+	–	+	–	+	–	+	–
	Str. cremoris H_{22}	+	–	+	–	+	–	+	±
	Strain 736	+	–	+	–	+	–	+	–
	Formamide extract:								
	Str. lactis OJ	+	–	+	–	+	–	+	–
	Str. lactis H_{14}	+	±	+	+	+	–	+	–
	Str. cremoris HP	+	–	+	–	+	–	+	–
	Str. cremoris H_{22}	+	–	+	–	+	–	+	±
	Strain 736	+	–	+	–	+	–	+	–

'absorption', and in others five inoculations of living organisms grown in DLB were given after twelve inoculations of the 'absorbed' organisms. There were, however, no marked differences between the sera obtained by these methods, the modifications being apparently unnecessary if the 'absorption' process were carried out.

A number of specific group N precipitating sera have been prepared by means of the 'absorption' technique for a variety of freshly isolated and freeze-dried strains of *Str. cremoris*. Table 4 shows the results of absorption tests of two such sera, and of the *Str.*

lactis group sera OJ and H_{14}. (Similar tests of a further *Str. cremoris* serum of this series are given by Briggs (2).) The results leave no doubt as to the group specificity of the sera or, therefore, of the serological classification of *Str. cremoris*.

DISCUSSION

Str. lactis and *Str. cremoris* have been differentiated in various ways by a number of workers; recently Hirsch & Grinsted (4) have divided them by means of cultural, biochemical, physiological and agglutination tests, and by their antibiotic production, and Hunter (5) by bacteriophage action. The cultural, biochemical and physiological characteristics of *Str. cremoris* are known to vary under differing conditions, but the extent of this variation, and whether *Str. cremoris* represents a distinct and stable type, particularly serologically, remains to be determined.

However, in every strain of *Str. cremoris* used in this work, whether freshly isolated or freeze-dried, the antigen common to the streptococci of groups K and L was present; and we have been unable to produce group-specific sera with these strains unless they were first 'absorbed' with antisera of groups K and L. These observations do not apply to our strains of *Str. lactis* and, further, preliminary work on the typing of group N streptococci—reciprocal absorption tests of *Str. lactis* and *Str. cremoris* type sera—so far suggests that *Str. cremoris* is, at least serologically, distinct and stable.

Serologically, as well as biochemically, strain 736 does not possess the known characteristics of our *Str. cremoris* strains. Seelemann & Carstens (14) describe the production with 736 of a group serum by means of a suspension prepared in the 'usual' way (i.e. formalinized suspensions (13)); they also maintain that only a proportion of 'cremoris' strains will yield a group serum. In our experience only 'absorbed' *Str. cremoris* cells yield specific group sera, and all the 'cremoris' strains we have investigated behaved similarly in this respect. The observations of Seelemann & Carstens on strain 736 apply to *Str. lactis*.

Moreover, Sharpe (16) has produced a group N serum for strain 736 by the use of a suspension heated at 56° C. for 1 hr.—the method which we have found to be the most satisfactory in the case of *Str. lactis*. Seelemann & Carstens used formamide extracts, which Sharpe (15) and ourselves have found to be more effective with 'lactis' than with 'cremoris', and preliminary absorption tests with 'lactis', 'cremoris' and 736 type sera suggest that the latter organism more closely resembles in serological type *Str. lactis* (H_{14}) than *Str. cremoris*.

Our biochemical and serological evidence, direct and indirect, identifies strain 736 as *Str. lactis*; 'absorption' of *Str. cremoris* cells with antisera of groups K or L thus represents the only method at present available for the production of specific group N precipitating sera for *Str. cremoris*, and consequently for the unequivocal identification of that organism.

SUMMARY

1. Attempts to produce in rabbits group N sera for *Streptococcus cremoris*, using suspensions of living, disintegrated and heat-treated bacterial cells, yielded only non-specific products, cross-reactions occurring most commonly and intensively with HCl extracts of the streptococci of groups K and L.

2. The non-specific antibodies in these sera were completely removed by absorption with streptococci of group K or L, the group N reactions being only partially impaired.

3. In order to inactivate, before immunizing rabbits, that antigen in the 'cremoris' cells responsible for stimulating the production of non-specific antibodies, the organisms were 'absorbed' with an excess of group K or L antiserum.

4. With suspensions of organisms treated in this way, potent group N specific precipitating sera were obtained for both freshly isolated and freeze-dried strains of *Str. cremoris*.

5. The identity of *Str. cremoris* with serological group N is thus established.

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474. A NOTE ON THE SEROLOGICAL CLASSIFICATION OF *STREPTOCOCCUS DIACETILACTIS* (MATUSZEWSKI *ET AL.*)

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Streptococcus diacetilactis, isolated from spontaneously soured potato mash, was first described by Matuszewski, Pijanowski and Supińska in 1936(3). Its essential features included the production of large amounts of lactic acid and volatile products (including diacetyl), and it differed from the 'lactis-cremoris' group primarily in the production of carbon dioxide in milk, the utilization of citrate and in its vegetable origin. More recently, in his investigation of certain diacetyl-producing and citric acid-fermenting streptococci from milk and from starter cultures, Swartling(5) showed that strains which resembled closely the *Str. diacetilactis* of Matuszewski *et al.* belonged to serological group N. In

Table 1. *Reciprocal absorption tests with group N sera*

	Group N sera											
	Str. lactis OJ				*Str. cremoris* HP				*Str. 'diacetilactis'* C26/40 (Swartling)			
		Absorbed with strain				Absorbed with strain				Absorbed with strain		
HCl extract of	Unabsorbed	OJ	HP	No. 2	Unabsorbed	OJ	HP	No. 2	Unabsorbed	OJ	HP	No. 2
Str. lactis OJ	+	−	−	−	+	−	−	−	+	−	−	−
Str. lactis Rice	+	−	−	−	+	−	−	−	+	−	−	−
Str. lactis M3	+	−	−	−	+	−	−	−	+	−	−	−
Str. cremoris HP	+	−	−	−	+	−	−	−	+	−	−	−
Str. cremoris RW	+	−	−	−	+	−	−	−	+	−	−	−
Str. cremoris J	+	−	−	−	+	−	−	−	+	−	−	−
Str. diacetilactis no. 2	+	−	−	−	+	−	−	−	+	−	−	−

an addendum to his paper, Swartling states that this observation has been confirmed by me with a freshly isolated strain of *Str. diacetilactis* (no. 2) received from Prof. Pijanowski and Dr Supińska; the purpose of this Note is to record the experimental evidence for that statement.

The materials and methods used were similar to those of Briggs & Newland(2) in their work on the serological classification of *Str. cremoris*, and to those of Swartling's own work. It is necessary to give here only the results of precipitation and reciprocal absorption tests with *Str. diacetilactis* no. 2 and the specific group N sera for *Str. lactis* OJ (see Shattock & Mattick(4)), *Str. cremoris* HP (see Briggs & Newland(2)), and the 'diacetilactis-like' streptococcus C26/40 (see Swartling(5)).

Briggs(1) and Briggs & Newland(2) have shown that *Str. cremoris* belongs with *Str. lactis* in group N, and the table shows the serological identity of *Str. diacetilactis* no. 2 with these organisms in that group. It may be suggested that, as was the case with *Str. cremoris*, a specific group serum should be prepared for *Str. diacetilactis* no. 2 before it is finally classified in group N; however, this is now unnecessary in view of the complete

similarity between this organism and Swartling's strains, against which specific group N sera have already been prepared. (5)

As with *Str. cremoris*, serological tests are necessary to determine the type specificity of *Str. diacetilactis*.

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475. STUDIES ON VARIATIONS IN THE SOLIDS-NOT-FAT CONTENT OF MILK

V. VARIATIONS DUE TO WINTER FEEDING PRACTICE

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INTRODUCTION

It was shown by Bailey (1) that at certain times of the year a statistically significant decline from year to year had occurred in the solids-not-fat fraction of the milk from the Institute herd of cows. The design of that survey materially reduced the number of possible causes of the decline, and the suggestion was made that changes in feeding practice might well be responsible. The present investigation was planned to reveal any such changes and, if found, to evaluate their effect. The data collected for this purpose was suitable for use in a wider field than was necessary for the achievement of these limited aims, so the investigation was extended to include an assessment of the effect of certain food qualities on milk yield and on milk quality.

STATISTICAL METHOD

Limitations of the data available for this study imposed strictures on the design of the investigation. The absence of complete feeding records during the grazing season restricted the study to changes in winter feeding practice only; further, the use of manufactured cattle cubes whose composition was not known, rendered the feeding records of many cows unsuitable for inclusion in the study. Therefore the study was restricted to records of cows that calved for the first time during the years 1935–46, and whose winter concentrate rations had been mixtures of grains and cakes compounded on the farm.

In order to obtain a sufficiently large number of suitable records it was necessary to include figures from two months of the year; February and March were selected, first because the downward trend in the solids-not-fat fraction of the milk was well marked during these months (Bailey (1)), and secondly because the rate of fall in both months was almost equal. The period of two months was not extended as the gain in numbers for the

study would in any case have been small, while the loss of uniformity in the feeding practice might have been large.

Lists were prepared of cows in their second to sixth month of lactation together with records of their milking performance and details of the foods they had received. In general, records from the month of February were chosen, but if a cow had been given manufactured cattle cubes in that month but not in March, the records for March were used. Each cow contributed figures from either February or from March, never from both months; each month contributed an approximately equal number of records.

Table 1. *Types of foods given to the cows during the twelve years of the survey*

	1935	1936	1937	1938	1939	1940	1941	1942	1943	1944	1945	1946
Barley meal	—	—	—	—	—	—	—	—	—	—	16½	20½
Bean meal	—	—	—	—	—	—	—	—	—	9½	20	3½
Beet pulp, dried	—	—	—	—	16	—	—	—	14	—	—	—
Bran	—	—	—	—	—	—	—	—	—	—	—	2½
Coconut cake	—	—	—	—	—	—	—	—	—	12½	—	—
Coconut meal	—	—	—	—	—	—	25	—	—	—	—	—
Cottonseed cake, dec.	2	—	—	—	—	—	—	—	—	—	—	—
Cottonseed meal	—	—	—	—	—	—	10	5	—	—	—	—
Grains, brewers' dried	30	4½	—	—	—	31	20	—	—	—	—	14½
Groundnut cake, dec.	2	17	16	—	16	7½	—	—	30	12½	3½	4
Groundnut meal	—	—	—	11½	—	—	—	5	—	—	—	—
Hominy chop	22	4½	—	—	—	—	—	—	—	—	—	—
Linseed, crushed	—	—	—	—	—	—	10	—	—	—	—	—
Linseed cake	—	1½	—	—	—	—	—	—	—	—	—	—
Maize meal	—	28	22½	—	—	—	—	—	—	—	—	—
Maize, flaked	3½	—	2	37	14	—	—	—	—	—	—	—
Maize germ cake	20	—	—	—	—	—	—	—	—	—	—	—
Maize germ meal	—	—	—	—	14	31	—	—	—	—	—	—
Maize gluten feed	3½	8	—	—	—	21½	—	20	7	12½	—	—
Molasses	—	7½	10	—	—	—	—	—	—	—	—	—
Oats, crushed	7	1	—	—	20	—	30	41	42	50	60	22½
Palm kernel cake	—	—	—	—	—	9	5	—	2	—	—	—
Pea meal	—	—	—	—	—	—	—	—	—	3	—	—
Pollards	—	28	12	44½	—	—	—	—	—	—	—	20½
Rice bran	—	—	22½	—	—	—	—	—	—	—	—	—
Rice meal	—	—	—	5½	—	—	—	—	—	—	—	—
Soya-bean meal	10	—	15	1½	—	—	—	8	—	—	—	—
Wheat, flaked	—	—	—	—	20	—	—	—	—	—	—	—
Wheat, tail	—	—	—	—	—	—	—	21	—	—	—	—
Yeast	—	—	—	—	—	—	—	—	5	—	—	2
Percentage of P.E. supplied by roughage	55	40	63	63	47	41	54	91	59	80	54	51
Percentage of S.E. supplied by roughage	32	45	56	45	38	76	58	86	70	51	42	42

The cows' rations were calculated as daily means from the monthly total. In this way it was thought to minimize any bias due to very short-term changes in the rations and to give a more representative picture of the types of foods given. Lists of the concentrate foods and their quantities expressed as percentages of the total weight of concentrates used for the cows in each year are presented in Table 1. The percentages of protein and starch equivalents of the total ration provided by home-grown roughage foods are included in the same table.

Details of the cows' rations were converted into terms of dry matter, dietary fat, protein and starch equivalents, all measured in pounds. The conversion factors used were obtained from the tables in *Rations for Livestock* (Woodman (2)). Of these factors the one most liable to error was that used for hay; after considerable thought it was decided to regard each year's hay to be of equivalent quality, unless the records gave specific advice on this score, and that the hay should be rated in the tables as 'poor'. This course was

followed owing to the absence of chemical analyses of many of the samples of hay and because the results of the analyses that had been made showed that the low rating for the hay was probably the most accurate. Of the two measurements of milk quality, the solids-not-fat percentages were corrected for stage in lactation according to the factors used by Bailey (3). The milk-fat percentages were not corrected for stage in lactation owing to the absence of exact knowledge of the variation in this fraction due to that cause. However, it is known that during the stage used, the variations are not large. The records of milk yields were accepted unaltered because changes in this measurement produce changes in the rations, and any adjustments for stage in lactation would defeat the object of the study.

Table 2. *Average values and the changes in them due to time*

Daily milk yield (lb.) $= c$. S.N.F. % $= d$. Milk-fat % $= f$.
Dry matter (lb.) $= x_1$.
Protein equivalent (lb.) $= x_3$. Starch equivalent (lb.) $= x_4$.
Time (years) $= t$.

	Average values	Regression coefficients measuring yearly change in value from 1935 to 1946
$\bar{c}$	$21{\cdot}11 \pm 0{\cdot}610$	$b_{ct} = -0{\cdot}299 \pm 0{\cdot}175$
$\bar{d}$	$9{\cdot}25 \pm 0{\cdot}031$	$b_{dt} = -0{\cdot}024 \pm 0{\cdot}0086$*
$\bar{f}$	$3{\cdot}79 \pm 0{\cdot}051$	$b_{ft} = -0{\cdot}0072 \pm 0{\cdot}0156$
$\bar{x}_1$	$26{\cdot}40 \pm 0{\cdot}051$	$b_{x1t} = +0{\cdot}478 \pm 0{\cdot}590$
$\bar{x}_2$	$0{\cdot}49 \pm 0{\cdot}0247$	$b_{x2t} = -0{\cdot}0134 \pm 0{\cdot}007$
$\bar{x}_3$	$1{\cdot}93 \pm 0{\cdot}061$	$b_{x3t} = -0{\cdot}0061 \pm 0{\cdot}018$
$\bar{x}_4$	$13{\cdot}14 \pm 0{\cdot}290$	$b_{x4t} = +0{\cdot}0544 \pm 0{\cdot}027$

* Judged significant when $P = 0{\cdot}05$.

When the conditions of selection and modification of the data had all been satisfied, it was found that sixty-one cows could contribute information to the study; for each animal there were records of milk yield, milk-fat and solids-not-fat percentages, dry matter, dietary fat, protein and starch equivalents. Mean figures from the sixty-one figures were calculated for each of these, and estimates of the extent to which the means changed during the twelve years of the survey were made by the calculation of linear regression coefficients. The results of these two sets of calculations are given in Table 2.

The way in which the values of the milk yield, milk-fat and solids-not-fat depend on the values of each other and on those of the other four measurements was calculated in two ways, first by regression coefficients and secondly by partial regression coefficients; these were obtained by the solution of the inverse matrix, using the square-root method introduced by Dwyer (4), followed by the construction of the 'c' matrix from which the partial regression coefficients were obtained. The results of these extended calculations are shown in Table 3.

RESULTS

There were very marked changes in the feeding practice of the cows under investigation (Table 1). Unfortunately, the regularity of the changes measured here was disturbed by the inclusion of data from experimental animals. These did not always receive rations typical of non-experimental cows in the same year.

From 1939 there was a considerable increase in the proportion of home-grown concentrate foods. This was chiefly in the form of crushed oats, but barley, beans and peas all helped to provide for the cows' needs. By 1945 no less than 96% of the concentrate rations of the cows studied were home grown. The home-grown corn crops took the place

of the imported foods, particularly the maize meal and flaked maize, which were not available during the war. This change led to an increase in the weight of food necessary to supply the same amount of starch equivalent, which implies a fall in food quality.

The proportion of the protein and the energy value of the foods supplied by the roughage fraction was considerably higher during the war years (Table 1). When measured as a progressive change the trend is not very regular owing to the use of figures from experimental rations in some years. The increasing dependence on roughage foods is another reason why it is true to say that the quality of the rations fell as a result of war-time conditions.

Table 3

Milk yield (lb.) $=c$. Solids-not-fat percentage $=d$. Milk-fat percentage $=f$. Dry matter (lb.) $=x_1$.
Dietary fat (lb.) $=x_2$. Protein equivalent (lb.) $=x_3$. Starch equivalent (lb.) $=x_4$.

Regression coefficients

Milk yield the dependent variate	S.N.F. % the dependent variate	Milk-fat % the dependent variate
$b_{cd} = -3{\cdot}524 \pm 2{\cdot}494$	$b_{dc} = -0{\cdot}009 \pm 0{\cdot}007$	$b_{fc} = -0{\cdot}032 \pm 0{\cdot}010^{**}$
$b_{cf} = -4{\cdot}628 \pm 3{\cdot}069$	$b_{df} = +0{\cdot}395 \pm 0{\cdot}067^{**}$	$b_{fd} = +1{\cdot}037 \pm 0{\cdot}170^{**}$
$b_{cx_1} = +0{\cdot}036 \pm 0{\cdot}145$	$b_{dx_1} = -0{\cdot}007 \pm 0{\cdot}007$	$b_{fx_1} = -0{\cdot}004 \pm 0{\cdot}012$
$b_{cx_2} = +14{\cdot}262 \pm 2{\cdot}624^{**}$	$b_{dx_2} = +0{\cdot}211 \pm 0{\cdot}163$	$b_{fx_2} = +0{\cdot}029 \pm 0{\cdot}266$
$b_{cx_3} = +5{\cdot}806 \pm 1{\cdot}048^{**}$	$b_{dx_3} = -0{\cdot}008 \pm 0{\cdot}066$	$b_{fx_3} = -0{\cdot}142 \pm 0{\cdot}103$
$b_{cx_4} = +1{\cdot}089 \pm 0{\cdot}227^{**}$	$b_{dx_4} = +0{\cdot}002 \pm 0{\cdot}014$	$b_{fx_4} = -0{\cdot}021 \pm 0{\cdot}022$

Partial regression coefficients

Milk yield the dependent variate	S.N.F. % the dependent variate	Milk-fat % the dependent variate
$b_{cd \cdot fx_1x_2x_3x_4} = -1{\cdot}729 \pm 2{\cdot}050$	$b_{dc \cdot fx_1x_2x_3x_4} = -0{\cdot}0237 \pm 0{\cdot}0092^{*}$	$b_{fc \cdot dx_1x_2x_3x_4} = +0{\cdot}7691 \pm 0{\cdot}2184^{**}$
$b_{cf \cdot dx_1x_2x_3x_4} = -1{\cdot}597 \pm 1{\cdot}257$	$b_{df \cdot cx_1x_2x_3x_4} = +0{\cdot}3222 \pm 0{\cdot}0486^{**}$	$b_{fd \cdot cx_1x_2x_3x_4} = -0{\cdot}0298 \pm 0{\cdot}0180$
$b_{cx_1 \cdot dfx_2x_3x_4} = -0{\cdot}933 \pm 0{\cdot}144^{**}$	$b_{dx_1 \cdot cfx_2x_3x_4} = -0{\cdot}0410 \pm 0{\cdot}0117^{**}$	$b_{fx_1 \cdot cdx_2x_3x_4} = +0{\cdot}0014 \pm 0{\cdot}0243$
$b_{cx_2 \cdot dfx_1x_3x_4} = +5{\cdot}785 \pm 2{\cdot}760^{*}$	$b_{dx_2 \cdot cfx_1x_3x_4} = +0{\cdot}2218 \pm 0{\cdot}1742$	$b_{fx_2 \cdot cdx_1x_3x_4} = +0{\cdot}4399 \pm 0{\cdot}3442$
$b_{cx_3 \cdot dfx_1x_2x_4} = +1{\cdot}895 \pm 1{\cdot}412$	$b_{dx_3 \cdot cfx_1x_2x_4} = +0{\cdot}0231 \pm 0{\cdot}0922$	$b_{fx_3 \cdot cdx_1x_2x_4} = -0{\cdot}0846 \pm 0{\cdot}1723$
$b_{cx_4 \cdot dfx_1x_2x_3} = +1{\cdot}764 \pm 0{\cdot}373^{**}$	$b_{dx_4 \cdot cfx_1x_2x_3} = +0{\cdot}2142 \pm 0{\cdot}0274^{**}$	$b_{fx_4 \cdot cdx_1x_2x_3} = +0{\cdot}0424 \pm 0{\cdot}0513$
$F = 24{\cdot}03^{**}$	$F = 12{\cdot}13^{**}$	$F = 2{\cdot}42^{*}$

* Judged significant when $P = 0{\cdot}05$.
** Judged significant when $P = 0{\cdot}01$.

The significance to be given to the regressions of the food qualities on years (Table 2) should be decided in the light of the known facts. Thus the decline in food quality already noted would certainly have led to an increase in the average dry-matter intake, and although the regression that measured this was not statistically significant, the collateral evidence shows that it must be considered real. The value of the regression indicated that the average daily intake of the animals studied increased by about half a pound for each year of the survey, so that the average daily dry-matter intake of a 1000 lb. cow in the last year of the survey would be about $(26{\cdot}4 + 6 \times 0{\cdot}48) = 29$ lb.

The increase in the use of rough foods, particularly straws, chaff and glumes, would be expected to decrease the amount of fat in the diet, and the calculations show that this took place (Table 2). The odds for the reliability of the regression, although not high enough to claim statistical significance, were nevertheless approaching that level, and the collateral evidence again shows that the regression should be considered real. The regression indicates that during the twelve years of the survey there was a reduction of about a quarter in the total dietary fat consumed by the cows.

The changes in the protein equivalent and the starch equivalent of the rations should be considered together. The actual intake of protein equivalent remained about constant, while the starch equivalent tended to increase, although not enough to make much

difference to the milk yield as estimated from the usual feeding standards. There is no good reason to relax the standards for significance of the trends in milk yield and milk quality as was the case in the trends for dry matter and dietary fat, for there is no collateral evidence for the fact of the changes. The assumption from the results must be that the decline in food quality accompanied by the possible overloading of the cow's digestive system was (*a*) sufficient to mask the expected increase in milk yield from the increased starch equivalent of the food, (*b*) actually decrease the solids-not-fat content of the milk and (*c*) make no difference to its milk-fat percentage.

The mean values (Table 2) of the milk yield and its quality are those to be expected from Dairy Shorthorn cows in about the fourth month of their first lactation. The mean dry-matter intake must, according to the usual standards, be judged about maximal for the type of cows considered, but evidence will be presented later to show that in fact the intake was too high.

The mean level of fat intake represents about 2% of the total ration. This is well within the range of levels from which changes in milk yield from changes in dietary fat intake can be expected (Maynard & McCoy (5)). It is perhaps rather a low level if compared with that contained in many pre-war rations, but there is no evidence that it was in any way inadequate.

The mean levels of protein equivalent and starch equivalent were both considerably above the levels suggested by the usual feeding standards. This is in part due to the use of the same maintenance ration for each cow in the herd; this ration was adequate for a mature 12 cwt. animal, and so provided too much for a 9 cwt. cow in her first lactation. The over-feeding was probably not so great as it seems, for the young animals were still growing and many were in calf. Therefore it is quite unwarranted to assume that the nutrients in excess of the standard (Woodman (2)), i.e. $(1{\cdot}93-1{\cdot}65)=0{\cdot}28$ lb. of protein equivalent and $(13{\cdot}14-11{\cdot}75)=1{\cdot}39$ lb. of starch equivalent, were all available for production. The standards of feeding actually used for the first calves cannot be calculated precisely, but in order to provide some measure for the discussion of the results, it has been estimated that they were fed 115% of the standard for both protein and starch equivalents.

The calculation of the relationships between milk yield, solids-not-fat and milk-fat percentages repeats on a within lactation basis what was done on a lactation basis by Bailey (6). The results, although expressed here as regressions instead of correlations, are the same. The values of the regressions should not be given much attention owing to doubt as to which should be the dependent and which the independent variate; the value of making the calculations lay not in the measurements of the degree of the relationships but in the elimination of the effects of the relationships, from the partial regression coefficients, so that changes, in say milk-fat percentage, could be studied without regard to concomitant changes in the solids-not-fat percentage. This is a refinement of some importance, for very often the yield of milk and concomitant changes in milk-fat percentage, particularly in short-term studies, have been explained in terms of the negative correlation between the two measurements.

The regression coefficients in Table 3 have been presented so that comparison may be made with the partial regression coefficients also given. The differences are discussed later.

It is important to remember when considering the results given in Table 3 that certain

assumptions were made to facilitate the calculations. The first of these was the assumption that the maintenance portion of the rations and that part that produced live-weight gain was of the same value for each cow studied, so that, as it was a common factor, it was legitimate to ignore it; owing to the absence of figures of the cows' weights it was not possible either to estimate the maintenance requirements or to evaluate the effects of their variation. The error arising from this assumption is believed to be very small, because the weights of the first calvers that were studied would not vary very much.

The second assumption that was made concerned the effects of each of the independent variates on the dependent variates; it was assumed that these effects were not influenced by time, in other words, that the effects in one year were in every way equivalent with those of each other year. The inclusion of data from experimental cows would not affect this.

A further consideration to be remembered when using the results arises from the well-known fact that biological production usually follows the law of diminishing returns (Jensen, Klein, Rauchenstein, Woodward & Smith(7); Headley(8)). This means that curvilinear regression is necessary to provide the best fit of any input and output relationship. In the study made here the small volume of data forced an assumption of linearity. This assumption strictly limits the use of the results to conditions closely similar to those obtaining in this survey; these are measured by the mean values (Table 2). At the mean values it is reasonable to suppose that the gradients of the linear regressions are very similar to those of the real curvilinear forms, so that the error arising from the assumption of linearity will be small at the mean, but will grow at an increasing rate as departures from the means are made.

There is a possibility that the gradients of the linear regressions are biased relative to the real regressions even at the mean value. Small differential digestibilities have been shown to exist between different rations and different planes of nutrition (Balch(9); Balch, Balch, Johnson & Turner(10)), which could produce such a distortion. Fairly drastic changes are necessary to effect measurable responses, so it is considered that for the comparatively narrow range of conditions studied here, these factors would not be important.

The partial regression coefficients (Table 3) show that milk yield varied directly with dietary fat and starch equivalent, but inversely with the dry matter. Solids-not-fat percentage varied directly with starch equivalent but inversely with dry matter. Milk-fat percentage did not vary with any attribute of the diet. To use the actual coefficients in an example: if the starch equivalent value of a lactating cow's ration was increased by 2 lb. and the dry-matter value by 4 lb., and if there was no change in the protein equivalent, the dietory fat, the milk yield and the milk-fat percentage, the solids-not-fat percentage would rise by $(+2\times0{\cdot}2142-4\times0{\cdot}041)\%=+0{\cdot}2644\%$. When using any one of the regression coefficients the effect of change in one independent variate on a dependent variate can be measured only if the mean value of the other independent variates remains constant.

The degree of success attained by the multiple regression equations that can be built up from the partial regression coefficients and the mean values, for a determination of the value of a dependent variate, can be measured by the F value of the ratio between the variance due to the multiple regression and that due to departures from it. When milk yield is the dependent variate, the F value is 24·03, which indicates that the milk yield

very largely depends on the independent variates. When solids-not-fat percentage is dependent the F value is 12·43, much less but still highly statistically significant. In the case of milk-fat percentage the F value is low and is hardly significant.

DISCUSSION

The change in solids-not-fat percentage due to changes in feeding practice

Although great changes in the feeding practice of the cows studied here were found, when the foods were all converted into measurement of dry matter, dietary fat, and protein and starch equivalents, no regularity in the changes could be admitted statistically; however, for reasons already given, the regressions of dry matter and dietary fat on years were considered to be real. When solids-not-fat percentage was the dependent variate, the partial regression coefficient, with dry matter the independent variate, was statistically significant, but when dietary fat was the independent variate the coefficient was not significant. If the first only of these partial regression coefficients is used in conjunction with the regression coefficient measuring dry matter on time, the resulting changes in solids-not-fat percentage per year is estimated at 0·019%, which is very close to the change (0·0237%) that actually took place. When all the coefficients are used, regardless of significance, the change is estimated at 0·0148%. These calculations provide very good evidence that at least a major part of the total decline in the level of solids-not-fat in the milk of the cows studied has been due to the fall in quality of the food given to the cows.

The effects of dry matter on milk yield and milk quality

The partial regression coefficients, with dry matter the independent variate, show beyond all reasonable doubt that for the cows studied, any increase in their dry matter intake produced a fall in milk yield and its solids-not-fat content but had no effect on its milk-fat content. In view of the many factors known to influence a cow's consumption of foods, and the fact that the dry-matter content is not a complete guide to the quantity a cow will eat, it may be that the figures of dry matter used in the calculation are representative of some other unknown food quality whose quantitative variations are substantially proportional to those of dry matter. This possibility can be disregarded as long as the rations considered are of the same general type as those studied here, i.e. winter rations, for the calculations show that a knowledge of the dry matter content of such rations is an adequate measure and can be used to assist in milk production.

The figures found here are the first published measurements of the effects of the levels of dry matter in cows' rations, and show at what low levels the bad effects can become operative. Boutflour has stressed the importance of dry-matter limitation for high-yielding cows, and has recommended that such animals should receive the pick of the available high-quality foods; the results found here show that such a course can be followed only at the expense of part of the lower yielding cows' output. It does not mean that it may not be profitable to follow such a course; an opinion on this cannot be given until the effects of dry matter on high yielding cows have been studied.

The differences between the regression and partial regressions of yield and solids-not-fat on dry matter call for some comment; in the regressions the effects of an increase in dry matter cannot be distinguished from the increase in starch equivalent that usually accompanies it. As has been shown, these two food qualities exert opposing effects, so it

is not surprising that the effect of the two together should be measured by a coefficient of low magnitude.

The effects of dietary fat on milk yield and milk quality

The effect of dietary fat on milk yield was found to be quite high; indeed, the actual value of the partial regression coefficient found here was rather more than twice that found by Loosli, Maynard & Lucas (11), although the fairly high error attached to this estimate indicates that the two estimates of the regression may not, in fact, be significantly different. The difference between the regression and partial regression coefficients was very large; the regression of yield on dietary fat made no allowance for the starch-equivalent content of the fat, so the value of the regression was grossly exaggerated.

The effect of dietary fat on milk quality was not determined by this analysis, the partial regressions were large but were attended by large estimates of errors. This does not allow an easy dismissal of the matter by an assumption of no effect. Garner & Sanders (12) have shown that different fats have different effects on the milk-fat content, and it may be that the solids-not-fat percentage is similarly affected. The rations studied in this survey were somewhat varied, so probably there was a considerable diversity of fats, although no exact information on this score was available. This would account for both the value and the large errors of the partial regressions. The errors might have been reduced if a sufficiently large mass of data had been available, so that different fractions of the total dietary fat could have been studied separately.

The effects of protein equivalent on milk yield and milk quality

The finding of Ezekiel, McNall & Morrison (13), that once a certain level of protein intake was realized any additions had no effect on milk quality, was borne out by the results found here, for in no case were the partial regression coefficients, with protein equivalent the independent variate, of high value or statistically significant. On the other hand, the regression coefficient of milk yield on protein equivalent was statistically significant and there was no good reason for this. The protein in excess of requirements could be used for its energy content, but the quantity so available would be too small to account for the high value of the regression coefficient. This unexplainable result must be attributed to chance and to the inadequacy of the method to deal faithfully with a complex question. There was no way of estimating the level at which additional protein fails to stimulate the milk yield and quality; the level would vary with those of the other factors involved.

The effects of starch equivalent on milk yield and milk quality

As should be expected, the starch equivalent value of the food was of very great importance to milk yield and its solids-not-fat content, and two of the partial regression coefficients involving starch equivalent achieve a high measure of statistical significance. The increase in milk yield due to increases in starch equivalent, was, of course, much less than would be the case if the mean level of starch equivalent input had been lower. This is in agreement with other workers (Jensen *et al.* (7), Headley (8)). The significant response in solids-not-fat percentage agreed with the work of Riddet, Campbell, McDowall & Cox (14) and Rowland (15). The non-significant response of milk-fat percentage to starch equivalent was not in agreement with the work of Steensberg (16) but agrees with the findings of Jensen *et al.* (7), who were unable to show any increase in milk-fat percentage. These discordant results are difficult to explain, and the results found here throw little

light on the problem; it may be that the usual response of a lactating cow to an increase in her starch equivalent ration is an increase in her milk yield and its solids-not-fat content, but under certain conditions not yet understood it seems likely that a proportion of the starch equivalent can be used for increasing the milk-fat content.

The conditions for maximum production

Although these calculations indicated the most desirable qualities for a ration, in practice economic considerations would be of overriding importance. To obtain maximum production from a cow of the type considered here, the dry-matter intake must be less than 26·4 lb. per day, the dietary fat intake greater than 0·49 lb. per day; the protein equivalent intake need not be as high as 1·93 lb. per day, but if the starch equivalent is increased in order to achieve maximum production it might become necessary to increase the protein equivalent level. The starch equivalent intake should be higher than 13·14 lb. per day. These ideals would be very difficult to achieve cheaply, and a majority of farmers would prefer to obtain less than the maximum possible milk yield from comparatively large quantities of an inexpensive ration. In these circumstances the limitation to production would be the capacity of the cow to consume food rather than her inherent ability to produce milk.

SUMMARY

During the twelve years from 1935 to 1946 the type of foods given to sixty-one Dairy Shorthorn first calvers during the months of February and March changed so that the dry-matter intake of the cows rose and the dietary fat fell, but there was little change in the Protein and Starch equivalent they received.

Milk yield varied directly with the amount of fat and starch equivalent in the diet and inversely with the dry matter.

The solids-not-fat percentage of milk varied directly with the amount of starch equivalent, and inversely with the dry matter content, of the diet.

The milk-fat percentage was not influenced by the foods given.

It was shown that the fall in the level of solids-not-fat in the milk was largely due to the increase in the dry matter intake of the cows.

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476. OBSERVATIONS ON THE INFLUENCE OF INHIBITORY SUBSTANCES PRODUCED BY THE LACTOBACILLI OF GRUYÈRE CHEESE ON THE DEVELOPMENT OF ANAEROBIC SPORE-FORMERS

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The importance of butyric acid-producing bacteria (genus *Clostridium*) in cheese-making is well known. Numerous faults in the ripening of cheese of the Swiss type have been attributed to the gas production or putrefying powers of these anaerobic spore-formers which in Cheddar cheese appear to be held in check by the salt concentration. Their sensitivity to salt is, however, dependent on pH. The optimum pH for growth is 6·8–7·2 (1). At the pH of young cheese 5·15 to 5·35, Zollikofer & Richard (2) found that most strains grew poorly in 4% NaCl, whereas at higher pH values growth was good. This may well account for the differences in the growth of clostridia in Swiss-type cheese and in Cheddar cheese. Not only is Cheddar cheese slightly more acid but the salt is evenly distributed throughout the cheese instead of being merely rubbed into the rind. But when Cheddar cheese is processed it may also develop these defects; the study of the butyric acid fermentation in cheese is obviously important.

Moreover, the development of clostridia in cheese is governed by many interesting factors. Thus Dorner (3) distinguishes between cheese defects caused by faulty manufacturing or curing and cheese faults caused mainly by bacteria. Only 5 cells/ml. in milk of *Clostridium butyricum* (Bergey) (referred to as *Bacterium amylobacter* by Dorner (1) and presumably synonymous with *Clostridium tyrobutyricum* of Van Beynum & Pette (4)) need be present to cause spoilage. With *Bacterium proteolyticum*, a non-sporing anaerobe, Dorner found that only 10 cells/ml. of milk were required to produce putrefaction of cheese (5). Other species, such as *Clostridium sporogenes*, may only develop under 'favourable conditions' not present in normal cheese.

It thus appears that numbers as well as the species of the contaminating anaerobe play an important part in the maturation of the cheese, but Richard (6) found in a large survey that freshly isolated strains of butyric acid bacilli were so variable as to make the identification of the more dangerous '*Cl. tyrobutyricum*' impossible. Further, it is well established that *Cl. sporogenes* frequently spoils processed cheese (7, 8), and that other anaerobic spore-formers may also occur (9). *Cl. perfringens* and *Cl. welchii* have been incriminated as the causative organisms in 'blown' unprocessed cheese (10, 11). It is thus difficult to assess the exact importance of the causative species. The difficulty is complicated by the confusion in the classification of these anaerobes. *Cl. tyrobutyricum* ferments lactate but not lactose according to one authority (4) and may ferment both according to another (6). It is not given species rank by Bergey, and in Prevot's manual (12) the organism is not mentioned.

It has been our experience, and that of most workers in this field (cf. ref. (13)), that experimental cheese heavily inoculated with a culture of *Cl. butyricum* may mature normally. Ritter also found that butyric acid bacteria may be isolated from normal Emmenthal cheese (13). It therefore appears that unknown factors in milk may dictate the course of the anaerobic fermentation. The importance of the optical activity of the lactate has been pointed out by Kiuru (14), and Foster & Wynne have shown that certain free fatty acids act as inhibitors to spore germination (15, 16, 17, 18). They concluded that 'dormancy' in spores is a function of the medium rather than of the spores themselves. The same workers have also shown that certain saturated fatty acids may inhibit spore formation. Starch has been shown to reverse the effect of fatty acids, and it is interesting to note that at a time when starch was fed to dairy cows in a district producing Gruyère cheese, there was a great increase in the incidence of 'blown' cheese (19).

On the other hand, it has been shown that growth and gas production of clostridia in Swiss-type cheese can be prevented by the use of a nisin-producing 'starter' (20). The nisin-producing organism was a strain of *Streptococcus lactis* (21) sufficiently heat-resistant to survive the cheese-making, although the numbers were reduced and the organism was less vigorous after the heat treatment (unpublished data). Recently, inhibitory lactobacilli have also been isolated from Gruyère cheese (22), and it is possible that one or both of the substances produced by organisms occurring naturally in Gruyère cheese may contribute to the suppression of the butyric acid fermentation.

A rational explanation for the absence of butyric acid fermentation in Swiss-type cheese when clostridia are known to be present in large numbers could be provided by a knowledge of the antibiotic phenomena occurring in the cheese. The work described in this communication was undertaken to test this hypothesis. The antibiotic techniques necessary for this study are described in some detail.

It is evident that lactic acid bacteria which produce inhibitory substances *in situ* may be convenient and inexpensive for controlling the butyric acid fermentation in processed and fresh Swiss-type cheese.

MATERIALS

Samples. Samples of cheese were obtained from factories situated in the district of Franche-Comté and all within a radius of 60 km. of Poligny (France). Some of the factories were in the plains, others on the first or the second plateau of the Jura mountains.

It was known that nisin disappears relatively quickly from cheese (20), and for this reason cheese 24 hr. old were usually sampled. A 15–20 g. bore was wrapped in tin-foil and tested in the laboratory either on the day of sampling or after storage overnight in the refrigerator. As a rule only one sample was taken from each cheese, although sometimes more than one cheese from the same factory was sampled.

Media. Meat-infusion broth and meat infusion agar were used for the cultivation and assay with the test organism *Staphylococcus aureus* (491).

Litmus milk was used for cultivation and assay with the test-organism *Streptococcus cremoris* (1P5).

Litmus milk was also used for the cultivation of the lactic organisms suspected of producing antibiotics, but a yeast glucose agar (Y.G.A.) containing yeast-extract (0·3%), peptone (Evans, 0·3%), meat extract (Lemco, 0·5%), glucose (1%), agar (2%) and thallous acetate (0·05%), was used for their isolation.

For the cultivation of the clostridia and for assays using these organisms a medium based on that of Bhat & Barker (23) and of Foster & Wynne (18) was devised. When it was desired to eliminate the lactic organisms from a mixed culture of lactic acid bacteria and clostridia, glucose was replaced by calcium lactate.

Composition of medium

	%
Yeast extract	1
Peptone (Evans)	0·3
Meat extract (Lemco)	0·3
Glucose	1
Na-acetate	0·5
Cystein	0·02
Soluble starch	0·1
Agar	0·05

METHODS

Isolation of clostridia. The technique of Weinzirl was used (24). 5 g. of the young cheese was ground with 40 ml. of warm Ringer solution in a sterile mortar. Some of this suspension was used for the isolation of lactic acid bacteria and the remainder distributed into five to six Weinzirl tubes, heated at 80° C. for 10 min. and incubated at 30° C.

Gas production, detected by the rise of the paraffin plug, was read at regular intervals (3, 6, 10 and 15 days). The volume of the gas and the number of positive tubes from each sample allowed an approximate estimate of the weight of the clostridial contamination to be made. The results were recorded as follows:

(1)	No gas production in any of the tubes	−
(2)	Doubtful or slight gas production in one or more tubes, reaction appearing after long incubation	±
(3)	Early and strong gas production in one tube only, others negative	+
(4)	Early and strong gas production in two tubes only, others negative	+ +
(5)	Early and strong gas production in three or more tubes	+ + +

Reactions (1)–(2) were considered negative, no growth of clostridia.
Reactions (3)–(5) were considered positive, growth of clostridia.

Antibiotic tests

Isolation of antibiotic producing strains. Colony spotting technique (C.S.T.).

This test (25) was modified to yield an estimate of the numbers of inhibitory lactic acid bacteria.

A small part of the suspension, aseptically prepared for the Weinzirl tests, was reserved for this test. The suspension was diluted in powers of 10 and dilutions chosen which were expected to yield 50–100 colonies when two drops were spread on Y.G.A. in Petri dishes. Duplicate plates were prepared and incubated at 37 and 45° C. The medium contained 1/2000 thallous acetate, which completely inhibited all organisms except lactobacilli and streptococci, colonies of which were clearly visible after 48 hr. incubation. A layer of meat-infusion agar buffered with 1% phosphates (pH 7·0) and inoculated with 1% of a 1/100 dilution of a 48 hr. culture of *Staph. aureus* was then poured on the top. The plates were placed in the refrigerator for 24 hr. and then incubated at 37° C. for 24 hr. The

addition of the buffer has no effect on the growth of the *Staphylococcus*, but by maintaining a constant pH throughout the medium it reduced the chances of false positive reactions due to lactic acid. The presence of the selective agent in the bottom agar layer was similarly without effect on the growth of the *Staphylococcus* as was shown in numerous control experiments. The refrigeration period permitted the diffusion of inhibitory substances from one layer of agar to the other. The time of refrigeration had an important effect on the reproducibility of the test.

On uncrowded plates colonies producing inhibitory substances were easily distinguishable by the clear zones around them. Usually from each plate five antibiotic colonies were picked into litmus milk.

The results were recorded thus:

No inhibitory colonies in the 10^{-3} dilution	−
Few inhibitory colonies in the 10^{-3} dilution	±
Numerous inhibitory colonies in the 10^{-3} dilution	+
Numerous inhibitory colonies in the 10^{-4} dilution	+ +
Numerous inhibitory colonies in the 10^{-5} dilution	+ + +

Confirmatory antibiotic tests with pure cultures

(*a*) *Diffusion assay.* Penicillin cups were placed on buffered meat-infusion agar inoculated, as described, with a culture of *Staph. aureus*. The clotted milk cultures were filled into the penicillin cups, refrigerated for 24 hr. and then incubated for 24 hr. at 37° C. Zones of inhibition were then measured.

(*b*) *Dilution assay with* Staph. aureus. Two-fold dilutions of the clotted milk culture were made in meat-infusion broth. The broth was inoculated with a 1/10,000 dilution of a 48 hr. culture of *Staph. aureus*. As there was no fermentable carbohydrate the lactic acid bacteria grew only slightly in the assay medium. Growth or absence of growth was recorded after 24 hr. incubation at 37° C.

(*c*) *Dilution assay with* Clostridium butyricum (7423). The technique of this assay was the same as for the dilution assay with staphylococci. Whole-milk cultures of lactobacilli were diluted in the medium in which the glucose was replaced by Ca-lactate to reduce the growth of the lactic acid bacteria. The medium was inoculated with a 1/1000 dilution of a young culture of the test organism and incubated at 30° C. for 24 hr. At a dilution of $<1/40$ only gas production could be determined, but at dilutions $>1/40$ the presence or absence of growth could be observed.

Tests on the whole cheese. 5 g. of cheese were emulsified without aseptic precautions in a tissue-blender with 20 g. of saline. Half of this suspension was brought to a pH below 3 with N-HCl and then brought to boil in a water-bath. This sample was then assayed for heat and acid-stable antibiotics (nisin) against *Streptococcus cremoris* (1P5) as the test organism using the rapid assay method of Hirsch & Wheater (25).

The remainder of the sample was placed in penicillin cups. After 24 hr. refrigeration followed by 24 hr. incubation the zones formed by the growth of *Staphylococcus aureus* were measured.

RESULTS

Clostridia. Forty-two factories were visited and fifty-one samples taken and examined by the Weinzirl test. Of these samples forty were contaminated, usually heavily, with clostridia (Table 1).

Inhibitory activity of the whole cheese. Of the fifty-one samples none was active by the nisin test, but nineteen gave zones of inhibition 10–22 mm. in diameter. It seems that none of the cheese contained nisin, the inhibitory action being due to a heat and acid-labile substance.

Inhibitory activity of the isolated strains (C.S.T.). The presence of inhibitory lactic acid bacteria was demonstrated in thirty-seven of the forty samples examined by this test. Table 2 shows that the activity of the samples varied considerably. About half the samples (nineteen) contained powerful inhibitory substances or large numbers of these organisms, since inhibition was clearly demonstrated at a dilution of 10^{-5}. At lower dilutions colonies of staphylococci were completely eliminated. Fifteen samples gave clear, though less marked inhibition, and with the remaining six samples inhibition was doubtful or absent.

From the thirty-four plates which gave strong inhibition 177 colonies were picked into litmus milk, and after two subcultures most of them were tested by the penicillin cup method. Fifty-five of the strains thus isolated were strongly inhibitory. The activity of some of these strains was confirmed by further tests with staphylococci and clostridia as the test organisms.

Table 1. *The distribution of clostridial contamination in cheese samples as measured by a modified Weinzirl test*

Extent of contamination	+++	++	+	±	−	Total
No. of samples	10	10	20	3	8	51

Table 2. *Intensity of antibiotic action of lactobacilli in cheese as measured by a colony-spotting technique* (C.S.T.)

Intensity of antibiotic action	+++	++	+	±	−
Number of samples	19	7	8	3	3
Numbers of colonies repicked		177			
Number of cultures active by penicillin-cup test		55			

Penicillin cup tests on isolated strains. The average diameter of the zones of inhibition was 13–15 mm., with a maximum for some strains of 30 mm. This confirms the strong inhibitory activity of some of these substances. That the zones of inhibition were not due to pH variation in the medium was shown by the following measurements:

Medium before *Staphylococcus* growth pH 7·0.
Medium, transparent zone of inhibition pH 6·95.
Medium, opaque zone of staphylococcal growth pH 7·54.

On the other hand, we have frequently observed that if by accident a drop of the clotted milk culture was placed directly on the agar far bigger zones of inhibition were obtained than with large volumes of the culture placed in penicillin cups.

Inhibition of staphylococci in liquid medium. With the antibiotic producing organisms grown in milk variable results were obtained probably because it was difficult to decide the exact point at which turbidity due to milk came to an end and the turbidity due to the growth of the test organism commenced.

In other experiments, to be reported in a separate communication, the producer organisms were grown in a broth supplemented with malt extract and Tween 80. Titration end-points were much easier to read and maximum titres of 1/1280 could be frequently obtained (cf. ref. (22)).

Inhibition of clostridia. Variable results were obtained in the first assays when a strain of *Clostridium sporogenes* was used as the test organism in meat-infusion medium. Later, when these experiments were repeated with *Cl. butyricum* in the lactate medium, variable results were still obtained. Eleven strains of the producer organisms were then grown at four different temperatures (Table 3). Strong inhibition of the test organism was now observed. Different strains produced the inhibitory substance at specific temperatures of incubation, although growth occurred at all temperatures between 30 and 50° C.

Table 3. *The inhibition of* Clostridium butyricum *at* 30° C. *by milk cultures of lactobacilli grown at different temperatures of incubation diluted to* 1/50

	Temp., °C. of incubation			
Culture no.	30	37	41	50
1	+	+	−	+
2	+	+	−	+
3	±	−	⊖	+
4	+	+	−	−
5	+	⊖	−	−
6	+	+	−	+
7	−	−	−	+
8	−	+	−	+
9	+	−	+	+
10	+	−	−	+
11	+	+	+	+

+ = growth and gas production by the test organism.
− = no growth and no gas production by the test organism.
⊖ = growth and gas production inhibited also at a dilution of 1/100.

Table 4. *Correlation between the antibiotic activity of whole cheese and C.S.T.*

		C.S.T.	
		+	−
Whole cheese	+	14	1
	−	20	5

Total 40 samples

Table 5. *Correlation between C.S.T. and the Weinzirl test for clostridia*

		Weinzirl test	
		+	−
C.S.T.	+	28	6
	−	3	3

Total 40 samples

Identity of the strains and of their inhibitory substances. All the fifty-five inhibitory cultures were Gram-positive, catalase-negative rods (genus *Lactobacillus*). There were no thermophilic streptococci among the strains isolated. The culture fluids contained inhibitory substances which were acid and heat labile, resembling lactobacillin (22) rather than nisin (21).

Examination of the data for correlation between various tests. Although the data are insufficient for a proper statistical examination it was evident that there was no correlation between the results of the inhibitory tests done on the whole cheese and C.S.T. It is clear (Table 4) that C.S.T. is a much more sensitive method of obtaining a measure of the inhibitory activity of cheese than the examination of the whole cheese. It was not surprising that no correlation could be found between the Weinzirl tests and the antibiotic activity done on the whole cheese, but when the more sensitive C.S.T. test was used good correlation between this and the Weinzirl test was found (Table 5).

DISCUSSION

Our evidence shows that some of the lactobacilli of young Gruyère cheese are powerful producers of inhibitory substances. If the results of the C.S.T. can be accepted as fairly representing the conditions in a young cheese, it would appear that almost all the cheese contain a large inhibitory flora. Further, there is high positive correlation between the C.S.T. test and the Weinzirl test for clostridia (Table 4), suggesting that these naturally occurring substances may be one of the factors inhibiting the development of the clostridia in young cheese. When the inhibition due to the whole cheese (not isolated strains) was determined, 30% of the samples were active, further strengthening our hypothesis.

The experiments recorded are directional rather than conclusive. Final conclusions must await the elucidation of the nature of the inhibitory phenomena involved and the ultimate effect of these substances on the clostridia. It is not yet known whether these organisms occur as spores, the germination of which is inhibited, or in the vegetative form, or both. However, the results in Table 3 show that as the cheese cool in the press, a variety of inhibitory strains become active because the optimum temperatures for the production of inhibitory substance(s) vary with different strains.

SUMMARY

1. The failure of clostridia to produce a butyric acid fermentation, when known to be present in Swiss-type cheese, is discussed.

2. It is suggested that inhibitory substances produced by the predominating lactic flora of young cheese are partly responsible for the suppression of the fermentation.

3. Details are given of the techniques used to investigate this supposition.

4. The evidence was obtained by examining fifty-one samples of Gruyère cheese, 78% of which were contaminated with clostridia. 85% of the samples contained inhibitory lactobacilli.

5. In 30% of the samples the inhibitory phenomena were sufficiently marked to give clear zones of inhibition against *Staphylococcus aureus* when a suspension of cheese was placed in penicillin cups.

6. Inhibition of *Clostridium butyricum* was demonstrated with pure cultures of lactobacilli. The cultures could grow at temperatures from 30 to 50° C., but were inhibitory only at certain temperatures of incubation which appear to be a strain characteristic.

7. The inhibitory substance(s) concerned is not nisin because it is acid and heat labile.

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477. ACTION INHIBITRICE DES STREPTOCOQUES PRODUCTEURS DE NISINE SUR LE DÉVELOPPEMENT DES SPORULÉS ANAÉROBIES DANS LE FROMAGE DE GRUYÈRE FONDU

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La formation de gaz dans la pâte du fromage fondu, au cours de la conservation, représente un défaut assez fréquent qui entraîne le gonflement ou même l'éclatement des emballages, notamment lorsque ces derniers sont constitués par des boites métalliques. Le gonflement fait considérer le fromage fondu comme impropre à la consommation; de plus il s'accompagne souvent d'une protéolyse qui accentue la mauvaise apparence du produit.

La formation de gaz et la protéolyse sont liées, dans la majorité des cas, au développement de germes sporulés anaérobies (clostridia) qui sont présents dans le fromage et survivent au chauffage pendant l'opération de fonte.

Les conditions dans lesquelles le défaut apparaît ont déjà fait l'objet de plusieurs études, notamment celles de Cziszar (1,2,3), Demeter et Janoschek (4), Godbersen (5): elles ont montré que la croissance des clostridia est influencée par la composition du milieu et la méthode de préparation des fromages fondus. Le pH et le rH de la pâte du fromage fondu (6,7,8), sa teneur en eau et en graisse (9), la température de cuisson et celle à laquelle le produit est maintenu pendant la conservation (10), enfin la nature des fromages—ou mélange de fromages—soumis à la fonte, sont autant de facteurs variables qui peuvent soit accélérer, soit retarder le gonflement des boites.

Dans la pratique on cherche, au moment de la fabrication, à se placer dans les conditions les moins favorables au développement des clostridia, mais l'expérience montre que les moyens habituellement mis en œuvre ne suffisent pas toujours à prévenir l'accident: avec certains fromages, très contaminés par les clostridia ou présentant simplement une composition favorable au développement de ces derniers, il est difficile d'obtenir un fromage fondu stable et d'empêcher les gonflements, surtout si le produit doit supporter des périodes de stockage prolongées à des températures voisines de 30°. Il semble que les fromages de gruyère fondus soient enclins, plus que d'autres, à présenter ce genre de défauts (11).

Récemment, l'un de nous (12), a montré que le développement des clostridia dans le gruyère pouvait être arrêté par l'adjonction au levain habituel d'une culture de *Streptococcus lactis* producteur de nisine. Ceci nous a conduits à étudier l'effet d'une addition de ces mêmes cultures au moment de la fabrication des fromages fondus.

Le but de notre travail était de voir si, dans les conditions pratiques de la fonte, la nisine était susceptible d'apporter une barrière supplémentaire venant s'opposer efficacement à la croissance des germes responsables du gonflement.

TECHNIQUE

Les essais ont été réalisés dans un petit pétrin chauffé à la vapeur permettant de traiter, lors de chaque opération de fonte, environ 4 kg. de fromage de gruyère préalablement râpé et bien mélangé.

La contamination du fromage par les clostridia était assurée par l'un ou l'autre des procédés suivants:

(*a*) L'addition d'une culture sporulée de clostridia au fromage, juste avant la fonte, réalisait une infection massive par ces microbes: nous avons utilisé la souche Cl. 6(12) ainsi que deux autres souches isolées de fromage fondu gonflé (3P, 4 Lgc).

(*b*) Dans l'un des essais la souche Cl. 6 a été inoculée, à forte dose, dans le lait de chaudière destiné à la fabrication du fromage et celui-ci, après maturation, a été fondu sans recevoir d'autre adjonction de culture de clostridia.

(*c*) Enfin nous avons employé également des fromages non inoculés artificiellement mais dont l'aspect révélait des caractères susceptibles de favoriser le gonflement ultérieur du fromage fondu. Il faut ajouter, qu'en l'état actuel de nos connaissances, le choix de ces derniers fromages ne repose pas sur des données précises et que le gonflement futur du fromage fondu demeure, pour une large part, imprévisible.

Nos divers essais ont comporté la préparation des pétrins suivants:

(*a*) Les pétrins servant de témoins recevaient les quantités d'eau et de sels de fonte (citrate de soude et acide citrique ou polyphosphates) nécessaires pour amener la teneur en substance sèche et le pH de la pâte à des valeurs déterminées et choisies à l'avance.

(*b*) Dans une autre série de pétrins, l'addition d'eau était remplacée par l'addition d'une culture sur lait de la souche de *Str. lactis* no. 12, productrice de nisine. La quantité de culture ajoutée, ainsi que la proportion de sels de fonte, étaient prévues de manière à conserver au produit final une teneur en substance sèche et un pH aussi voisins que possible de ceux des pétrins témoins.

(*c*) Nous avons préparé également des pétrins en remplaçant la culture de la souche no. 12 soit par du lait pasteurisé, soit par une culture sur lait de la souche de *Str. cremoris* 1P5 qui ne produit pas de nisine et sert d'organisme-test pour le dosage de cette substance. Notre but était de vérifier ainsi l'influence qu'une addition de lait pouvait exercer sur le développement des clostridia dans le fromage fondu.

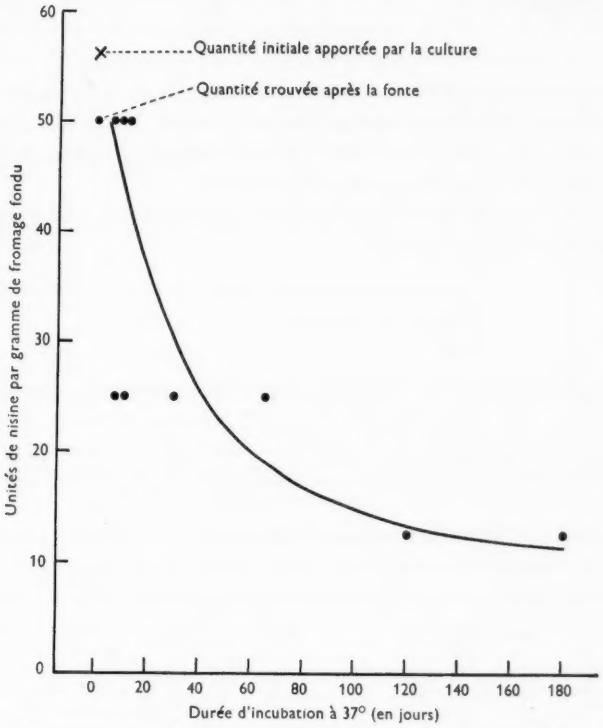
(*d*) Enfin, dans certains essais, nous avons ajouté, au lieu de la culture inhibitrice, une solution de nisine de façon à obtenir dans le fromage fondu, sans introduction de lait, des titres en nisine semblables à ceux que nous donnaient les cultures de la souche no. 12.

Après la fonte, le fromage encore liquide était aussitôt réparti en boites métalliques (boites 1/10ème basses de 90 g.) et en boites de Pétri que l'on remplissait aussi complètement que possible. Toutes étaient alors placées à l'étuve à 37°.

Des essais comparés à différentes températures nous ont montré qu'entre 30° et 37° la proportion des boites gonflées au bout d'un mois ne variait pas de façon sensible; à 45°, par contre, les gonflements se produisaient beaucoup plus lentement. Nous avons adopté 37° comme température de base pour l'épreuve de conservation.

Les examens étaient effectués chaque jour pendant toute la durée d'incubation: on

notait le bombement des boites métalliques et, dans les boites de Pétri, l'apparition de taches blanches, présentant souvent un centre noir et constituant les centres de développement des clostridia. La liquéfaction partielle du fromage (résultant de la protéolyse) était également visible à l'œil dans le cas des boites de Pétri, ou perceptible par simple agitation permettant de reconnaître, à l'ouïe, la présence de liquide dans les boites métalliques.



Graph 1. Teneur en nisine au cours de la conservation du fromage fondu à 37°.

On a remarqué, au cours du travail que, pour établir des comparaisons valables entre plusieurs essais, il était préférable de répartir le contenu de chaque pétrin dans un nombre relativement élevé de boites divisionnaires: 20 à 40 par exemple.

Le dosage de la nisine dans les cultures et dans les fromages a été effectué par une méthode de dilution avec *Str. cremoris* (1P5) comme organisme-test.

Nous avons vérifié qu'aucun des fromages (Gruyère) utilisés pour nos essais ne contenait de nisine.

RESULTATS

Les tableaux indiquent les résultats obtenus dans quatre séries d'essais (1–2–3–4).

Chaque série d'essais a été préparée en partant d'un seul mélange de fromages, râpé et bien homogénéisé. Par contre, ainsi que nous l'avons indiqué plus haut, les mélanges de fromages ont été différents d'une série à l'autre et l'aptitude au gonflement de ces divers mélanges s'est révélée, à l'expérience, comme assez variable; elle dépend sans doute plus de la composition des fromages et de l'activité des clostridia présents que du nombre total

de ces germes par gramme de fromage. En effet les résultats consignés dans le tableau 4 ont été obtenus avec des fromages non inoculés qui ont, en moyenne, gonflé plus vite que les fromages additionnés à forte dose d'une culture de clostridia (tableaux 1, 2 et 3). Dans le cas du mélange de fromages du tableau 2 les boites témoins (addition d'eau) ont gonflé à la même vitesse sans inoculation (A) ou avec inoculation de clostridia (B).

Des observations analogues aux nôtres ont été faites à ce sujet par différents auteurs et notamment par Ritter (13).

Action inhibitrice de la nisine sur les gonflements

L'action inhibitrice de la nisine est elle-même fonction, dans une certaine mesure, de la plus ou moins grande tendance au gonflement des fromages et probablement de la sensibilité des différentes souches de clostridia à l'antibiotique mais, dans l'ensemble, cette action est bien marquée pour les quatre séries d'essais.

Les résultats qui figurent dans les tableaux se rapportent à des fromages fondus présentant les caractéristiques suivantes:

teneur en substance sèche	44%
teneur en graisse	19%
pH	6,0

Une telle composition place déjà le fromage fondu dans une zone favorable au développement des clostridia. Les essais effectués à des pH plus bas ou avec des teneurs en substance sèche plus élevées (non rapportés ici) nous ont donné des résultats parallèles à ceux que nous exposons maintenant mais la vitesse moyenne de gonflement se trouve ralentie.

Tableau 1. *Effet d'une addition de culture de* Streptococcus lactis *no.* 12 *sur le gonflement des boites de fromage fondu après incubation prolongée à* 37°

(Les chiffres indiquent le pourcentage de boites gonflées pendant la période considérée).

Liquide ajouté	Eau	Eau	1P5 sur lait	No. 12 sur lait
Unités de nisine/g. de fromage*	0	0	0	50
Inoculation par les Clostridia	0	Cl. 6 + 3P + 4Lgc		
0–25 jours	0	100	63	0
25–50 jours	33	—	37	9
50–100 jours	33	—	—	9
100–150 jours	0	—	—	0
150–200 jours	0	—	—	9
% total de boites gonflées après 200 jours	66	100	100	27

* Titre en nisine trouvé dans le fromage aussitôt après la fonte.

Les chiffres du tableau 1 montrent que toutes les boites de fromage fondu ayant reçu une culture sur lait de 1P5 gonflent en moins de 50 jours, alors que 9% seulement des boites additionnées de la culture inhibitrice no. 12 gonflent pendant le même laps de temps. Après 200 jours d'étuve à 37° la proportion des boites gonflées, dans l'essai qui a reçu la culture inhibitrice, atteint 27%.

Sur le tableau 2, on voit que les boites E, additionnées d'une culture fortement inhibitrice (100 unités de nisine par gramme de fromage) ont gonflé dans la proportion de 2% après 15 jours à 37°, alors que 100% des boites 'témoins' A et B ont gonflé pendant cette même période. Après 60 jours à 37°, la proportion des boites gonflées du lot E ne dépasse par 5%.

Enfin (tableau 3) le fromage provenant d'un lait infecté par les clostridia (souche Cl. 6) a provoqué en 25 jours le gonflement complet de toutes les boites témoins, alors que l'on ne constate aucun gonflement dans le même fromage additionné d'une solution de nisine (50 unités par gramme de fromage). Après 60 jours, 15% seulement des boites additionnées de nisine étaient gonflées.

Tableau 2. *Effet d'une addition de nisine en solution aqueuse ou sous forme de culture* Streptococcus lactis *no.* 12 *sur le gonflement à* 37° C. *des boites de fromage fondu*

Essai ...	A	B	C	D	E
Liquide ajouté ...	Eau	Eau	Sol. aqueuse de nisine	No. 12 sur lait	No. 12 sur lait
Unités de nisine/g. de fromage ...	0	0	50	50	100
Inoculation par les Clostridia ...	0	Cl. 6 + 3P			
0–5 jours	0	0	0	0	0
5–10 jours	73	43	0	53	2
10–15 jours	27	57	15	25	0
15–20 jours	—	—	31	5	3
20–25 jours	—	—	8	0	0
25–30 jours	—	—	3	0	0
30–40 jours	—	—	3	0	0
40–50 jours	—	—	5	0	0
50–60 jours	—	—	5	0	0
% total de boites gonflées après 60 jours	100	100	70	83	5

Tableau 3. *Effet de la nisine sur le gonflement à* 37° C. *des boites de fromage fondu préparées avec un fromage provenant de lait infecté par Cl.* 6

Liquide ajouté ...	Eau	Solution aqueuse de nisine
Unités de nisine/g. de fromage ...	0	50
0–5 jours	0	0
5–10 jours	20	0
10–15 jours	43	0
15–20 jours	30	0
20–25 jours	7	0
25–30 jours	—	0
30–40 jours	—	8
40–50 jours	—	5
50–60 jours	—	2
% total des boites gonflées après 60 jours	100	15

Cette action spécifique de la nisine est d'autant plus remarquable que le lait ajouté au fromage fondu semble exercer le plus souvent une influence favorisante sur la production de gaz par les clostridia (tableaux 2 et 4).

Le tableau 4 montre que les deux lots de boites additionnés l'un de lait, l'autre de la culture de *Str. cremoris* 1P5 ont gonflé avec une vitesse maximale et d'ailleurs sensiblement égale. Par contre, les boites 'témoins' (fromage additionné d'eau) gonflent moins vite que les précédentes. Enfin les boites ayant reçu la culture de *Str. lactis no.* 12 ont gonflé les dernières. De même dans le tableau 2, on voit que le fromage D, additionné de culture no. 12 sur lait et titrant 50 unités de nisine par gramme, gonfle plus vite que le fromage C qui contient la même dose de nisine, mais apportée sous forme de solution aqueuse.

Tous ces résultats sont en bon accord avec les observations de Hood & Bowen (14) qui ont noté un meilleur développement des clostridia dans le fromage fondu additionné de lait écrémé ou de poudre de lait.

Toutefois, à partir du moment où une concentration suffisante en nisine est atteinte (fromage E: 100 unités par gramme, Tableau 2) l'action inhibitrice est assez forte pour compenser l'effet favorable résultant d'un apport de lait sur le développement des clostridia et arrêter pratiquement la croissance de ces derniers. Nous avons déjà noté que le mélange de fromages correspondant aux essais du tableau 2 gonflait à peu près à la même vitesse avec ou sans inoculation artificielle de clostridia et que l'aptitude au gonflement des témoins A et B était très marquée. Le résultat obtenu avec les boites E paraît donc significatif (Tableau 2).

Tableau 4. *Influence d'une addition de lait ou de lait acide sur le gonflement à 37° C. des boites de fromage fondu préparées avec un mélange de fromage n'ayant pas reçu une inoculation artificielle de clostridia*

Liquide ajouté ...	Eau	Lait	IP5 sur lait	No. 12 sur lait
Unités de nisine/g. de fromage ...	0	0	0	60
1 jour	0	0	0	0
2 jours	0	10	5	0
3 jours	5	65	60	0
4 jours	30	20	30	25
5 jours	60	5	5	15
6 jours	0	—	—	40
7 jours	5	—	—	15
8 jours	—	—	—	0
9 jours	—	—	—	5
% total des boites gonflées après 9 jours	100	100	100	100

Stabilité de la nisine dans le fromage fondu

On sait (15) que la nisine est thermostable en milieu acide: dans les cultures sur lait dont le pH final est de 4,6 elle résiste au chauffage à l'ébullition au bain-marie.

Avec les procédés de fonte utilisés pour le fromage de gruyère la température reste comprise entre 80° et 90° pendant environ 10 min., le pH du produit étant généralement situé à l'intérieur de la zone 5,6–6,2 (en moyenne 5,8).

Nous avons vérifié que malgré ce pH élevé, la proportion de nisine détruite au cours du chauffage dans le pétrin demeure assez faible, de l'ordre de 10% de la quantité initiale; le fromage fondu contient donc 90% environ de la nisine apportée par la culture et ceci même lorsque le pH est de 6,2 et la température de 90°.

Un chauffage ultérieur* du fromage fondu, effectué après la distribution en boites métalliques—25 min. au bain-marie bouillant—n'exerce pas non plus d'action destructrice appréciable sur la nisine.

Le taux de nisine dans le fromage fondu diminue lentement au cours de la conservation: le graphique I montre la disparition progressive de l'antibiotique dans les boites de fromage fondu maintenues à 37°.

On voit qu'après 180 jours d'étuve à 37° le fromage fondu contient encore le quart de la quantité de nisine présente aussitôt après l'opération de fonte.

Lors d'un essai antérieur (12) l'un de nous a observé une disparition complète de la

* Un tel chauffage est parfois utilisé dans l'industrie pour compléter la destruction des germes.

nisine 20 jours environ après la fabrication du fromage. La différence entre ce résultat et ceux que nous rapportons ici suggère que la stabilité de la nisine est peut-être fonction des phénomènes biologiques d'origine microbienne qui se succèdent au cours de la maturation normale du fromage. Dans le fromage fondu, ces phénomènes biologiques se trouvent arrêtés par suite du chauffage—à l'exception précisément de ceux qui peuvent être liés au développement accidentel des clostridia—et la stabilité de la nisine est meilleure.

CONCLUSION ET RÉSUMÉ

L'addition au moment de la fonte d'une culture sur lait de streptocoques inhibiteurs producteurs de nisine permet de réduire dans des proportions très notables les chances de développement des clostridia dans le fromage fondu infecté artificiellement par ces microbes ou provenant de fromages (Gruyère) suspects de gonflement.

La nisine ne subit pas de destruction importante au cours des opérations de fonte et sa stabilité pendant la conservation du fromage fondu est supérieure à celle que l'on a observée dans le fromage en cours de maturation.

Nous sommes heureux de remercier très vivement le Dr A. T. R. Mattick, Directeur-adjoint du National Institute for Research in Dairying, qui a bien voulu nous suggérer ce travail et prendre intérêt aux résultats que nous rapportons ici.

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637.352.2 (62)

478. HARD CHEESE MADE FROM EGYPTIAN COWS' AND BUFFALOES' MILKS

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(With 1 Figure)

CHEMICAL CHARACTERISTICS AND QUALITY

It is generally recognized by Egyptian cheese-makers and consumers that buffaloes' milk produces hard cheese of much lower quality than those made from cows' milk. The former cheese are usually harsh, flat-flavoured and dry. Buffaloes' milk, on the other hand, constitutes the major portion of the total milk supply of Egypt, and many small factories in the country use this milk in the manufacture of hard cheese. This, together with the high fat and solids-not-fat contents of buffaloes' milk, render improvement of quality highly desirable.

In the absence of any data on the hard cheese-making properties of buffaloes' milk, or on the composition of cheese made from it, a preliminary study must precede any attempt to secure improvement. The first part of this paper, therefore, deals with the results of twenty experiments which were carried out to investigate the various aspects of hard cheese-making from this milk, for comparison with cheese made from Egyptian cows' milk.

EXPERIMENTAL

The investigation was divided into two sections. The first section consists of ten experiments carried out on three types of hard cheese, namely, Cheddar, Derby and Manfis. Each of the first two types was made in three experiments, while the Manfis type was made four times. In all experiments one cheese was made from each of the morning bulks of cows' and of buffaloes' herd milk. The two herds belong to the Faculty and are well managed and the animals were free from clinical disease.

Calculated quantities of each of the two milks were taken, to yield the same weights of curd after the Cheddaring process. After straining the two lots into two separate vats, Cheddar or Derby cheese were made using normal methods of manufacture (1,2), while the Manfis type was made according to the original method described by Seleim (3). The two batches of cows' and buffaloes' milks were dealt with similarly (Table 1), and the resultant curds were put to press under equal pressure for each type. Cheese were also ripened in the same ripening room at 55–60° F. and 85–90 % humidity. The ripening period was considered to be 2 months for the Derby type, $2\frac{1}{2}$ months for the Manfis and 3 months for the Cheddar. Fat and acidity were determined in milk and whey, while moisture, fat, total N, soluble N, non-protein N, salt and acidity were determined in mature cheese.

The second section comprised two sets of five experiments each. In each experiment two Derby cheese were made; one being made from cows' milk and the other from buffaloes' milk. The only difference between the two sets was that, with the first set, the two curds were milled after Cheddaring for the same time, while with the second set,

buffaloes' milk curd was left until it gave threads of approximately the same length on the hot iron as those given by cows' curd, i.e. $\frac{1}{2}$ in. It was found that, in the latter set, buffaloes' milk curd required a much longer Cheddaring time than that of cows' (Table 2). The cheese were all ripened in the room used for those described in § I on p. 197.

Milk and whey were analysed in detail, but, owing to the importance of acidity and fat estimations in cheese-making, the discussion will be limited to these results. Acidity, moisture, total N, soluble N and non-protein N were determined in green cheese when taken out of press, and also at the end of the 1st, 2nd, 4th, 6th and 8th weeks, while fat and salt contents were only determined in green cheese and at the end of the ripening period. All cheese were also graded at the end of each experiment by three scorers, sixty points being allowed for flavour and aroma, and forty for body and texture.

ANALYTICAL METHODS

Sampling

Milk and whey. Milk samples were taken from the cheese vat after thorough mixing, while whey samples were taken from the whey collected after drawing off and during the Cheddaring step. The chemical analyses were carried out immediately.

Cheese. Using a sterile cheese-borer, three plugs were taken from each cheese extending from one surface to another except with the Cheddar type, where the plugs were extended to a depth of 10 cm. One of these plugs was taken about the centre of the cheese, the second about one inch from the outer edge, and the third at a point half-way between the two. The holes were immediately filled with sterile paraffin wax and the cheese replaced in the ripening room until used for the next examination. Cheese samples were quickly minced, placed in a glass weighing bottle and analysed forthwith.

Analysis of milk and whey

Acidity. Determined by the standard titration method, the results being expressed as percentage lactic acid.

Fat. Determined by the original Gerber method.

Analysis of cheese

Fat, moisture, acidity and salt. For these estimations the methods described by the British Standard Institution were used (4).

Total N. About 0·2 g. of the cheese sample was accurately weighed and digested by the Kjeldahl method. The procedure then continued as usual.

Soluble N. About 5 g. of the sample were accurately weighed in a 100 ml. beaker, and well ground with acid-digested sand and 25 ml. of distilled water at 50° C. The mixture was well stirred three times and left for 30 min. to settle. The clear decanted liquid was filtered into a 250 ml. graduated flask. The extraction and filtration were repeated three more times. The contents were completed to the mark with distilled water, well mixed and 25 ml. taken for the Kjeldahl estimation.

Non-protein N. About 2 g. of the sample were weighed in a 100 ml. beaker and well ground with acid-digested sand. Forty ml. of distilled water were added and the mixture stirred. Ten ml. of 20 % trichloracetic acid were then well mixed with the contents, and the mixture warmed to 70° C. with continuous stirring. It was then cooled to room temperature, filtered and washed with 1 % acid until 100 ml. of the filtrate were obtained, of which 25 ml. were taken for the Kjeldahl estimation. (5)

RESULTS AND DISCUSSION

Section I

Cheese yield and fat losses

The cheese records show (Tables 1 and 2) that cows' milk contains less fat than that of buffaloes'. According to several authorities(6,7), this would explain the higher cheese yield from the latter milk as shown in Table 3.

The acidity of cows' milk (Table 1) is usually higher than that of buffaloes' at the start of the manufacturing process in spite of its lower solids-not-fat content. The interval from the start of the process to milling is much longer for buffaloes' milk because of its lower acidity, and because of the slower rate of acid development, which is usually associated with milks of higher buffer value(8). Buffaloes' whey is of lower acidity at cutting than that of cows', and the subsequent rate of acid development is also much slower.

The loss of fat, in whey, is much higher in buffaloes' milk whey than in that of cows' (Table 1). This may be attributed to the higher fat content and/or the lower casein/fat ration of the former milk(6) and possibly to the higher proportion of large-sized fat globules in buffaloes' milk. In this study losses were higher with the Manfis type than with the others. As that cheese is more vigorously cut, and is also scalded to a higher temperature, this higher loss is expected. Its whey-fat content, therefore, resembles that of Swiss cheese which contains from 0·5 to 1·0 % fat as shown by Sammis(7). It must be mentioned, however, that the figures obtained in this study for the fat content of cows' whey from Cheddar and Derby cheese are noticeably lower than those given by other workers(9,10).

Composition of cheese

With both kinds of milks, the most distinct differences in the composition of the three types (Table 4) is that the Cheddar type contains less moisture than the two other types made from the same milk; consequently it is richer in fat and total N. Its acidity is also the highest, thus causing ripening, as judged by the chemical estimations of soluble and non-protein N contents, to proceed further than in the case of the Derby type in spite of the latter's higher moisture content. On the other hand, the higher soluble N and non-protein N contents of the Manfis type compared with the other two is explained by the fact that its moisture content is higher than that of the Cheddar type, together with the longer ripening period as compared with the Derby type.

The salt content of the Manfis type is higher than that of the others, because in the manufacture of this type after the first salting of the curd, the cheese is again soaked in 10 % salt solution for 24 h. after removal from press.

Comparing the composition of cheese made from Egyptian cows' milk with that of cheese made abroad from the same kind of milk, the results of the present study agree with those given by some investigators, but they vary widely from those given by others. Nicholls's results(11) for Cheddar cheese show higher moisture and lower fat contents —36·69 and 31·82 % respectively. On the other hand, his results for the moisture and fat contents of Derby cheese—39·95 and 30·59 % respectively—are remarkably close to those of Egyptian cows' milk Derby cheese. Davel's results(12) for South African Cheddar cheese are also in close agreement with the results given for the Cheddar type in the present study.

Table 1. *Details of the cheese-making process* (§ I)

Table 1. *Details of the cheese-making process* (§ I)

	Cheddar				Derby				Manfis			
	Cows		Buffaloes		Cows		Buffaloes		Cows		Buffaloes	
	Average	Range	Average	Range	Average	Range	Average	Range	Average	Range	Average	Range
Quantity of milk (lb.)	120	—	80	—	95	—	65	—	90	—	60	—
Fat in milk (%)	3·40	3·20–3·60	6·03	5·70–6·40	3·07	2·70–3·40	5·65	5·60–5·70	3·13	2·90–3·30	6·05	5·40–6·50
Renneting temp. (° F.)	85·5	85–86	85·5	85–86	85	—	85	—	93	—	93	—
Time of milk ripening (min.)	93	40–195	93	40–195	37	30–40	37	30–40	38	30–50	38	30–50
Initial acidity of milk (%)	0·21	0·20–0·22	0·20	0·18–0·21	0·208	0·205–0·210	0·200	0·185–0·210	0·205	0·185–0·225	0·194	0·170–0·210
Acidity at renneting (%)	0·218	0·210–0·230	0·212	0·205–0·220	0·210	—	0·198	0·190–0·210	0·211	0·190–0·225	0·199	0·175–0·220
Time of coagulation (min.)	48	35–60	48	35–60	57	50–60	57	50–60	48	45–50	46	40–50
Fat in whey (%)	0·20	0·10–0·25	0·70	0·60–0·80	0·15	—	0·53	0·45–0·65	0·38	0·30–0·50	1·06	0·95–1·20
Acidity at cutting (%)	0·135	0·130–0·140	0·115	0·110–0·125	0·135	0·130–0·140	0·112	0·110–0·115	0·136	0·130–0·145	0·116	0·095–0·130
Scalding temp. (° F.)	102	—	102	—	95	—	95	—	103	—	103	—
Time of scalding (min.)	60	—	60	—	60	—	60	—	30	—	30	—
Time of pitching (min.)	108	45–190	163	60–235	7	5–10	132	65–200	51	10–105	278	85–335
Acidity of whey at drawing off (%)	0·190	—	0·190	—	0·145	0·140–0·150	0·145	0·140–0·150	0·150	—	0·150	—
Time of cheddaring (min.)	168	135–205	192	145–280	135	120–150	165	150–180	No cheddaring		No cheddaring	

Table 2. *Details of the cheese-making process* (§ II)

	First set				Second set			
	Cows		Buffaloes		Cows		Buffaloes	
	Average	Range	Average	Range	Average	Range	Average	Range
Quantity of milk (lb.)	95	—	65	—	95	—	65	—
Fat in milk (%)	3·35	3·10–3·55	5·96	5·60–6·60	3·37	3·10–3·60	5·54	5·30–5·80
Renneting temp. (° F.)	85	—	85	—	85	—	85	—
Time of milk ripening (min.)	28	5–45	33	30–45	38	30–45	38	30–45
Initial acidity of milk (%)	0·216	0·210–0·225	0·205	0·190–0·215	0·205	0·190–0·215	0·203	0·190–0·210
Acidity at renneting (%)	0·219	0·210–0·230	0·207	0·190–0·225	0·206	0·190–0·215	0·203	0·190–0·210
Time of coagulation (min.)	62	50–85	59	50–70	55	50–60	55	50–60
Fat in whey (%)	0·14	0·10–0·20	0·55	0·30–0·80	0·14	0·10–0·20	0·44	0·35–0·60
Acidity at cutting(%)	0·138	0·125–0·150	0·124	0·105–0·135	0·135	0·130–0·145	0·116	0·105–0·130
Scalding temp. (° F.)	95	—	95	—	95	—	95	—
Time of scalding (min.)	60	—	60	—	60	—	60	—
Time of pitching (min.)	8	5–15	165	120–290	23	5–75	109	35–170
Acidity of whey at drawing off (%)	0·147	0·140–0·155	0·147	0·140–0·155	0·144	0·140–0·155	0·144	0·140–0·155
Time of cheddaring (min.)	128	120–145	138	120–165	133	110–165	184	155–240

Allen & Knowles (13), in their study on Cheddar cheese, obtained for normal cheese an average acidity of 3·45 % in one experiment, and 2·70 % in another after 25–26 weeks. Davies, Davis, Dearden & Mattick (14), studying the effect of chemical substances on the ripening of the same type of cheese, showed that the average acidity of the control cheese was 1·93 % after 64 days. The average acidity of cows' Cheddar cheese given in this study is higher than that given by Davies *et al.*, while it agrees reasonably well with that given by Allen & Knowles.

Van Slyke (6) showed that the total water-soluble N expressed as percentage of total N was 21·44 % after 1½ months and 31·00 % after 3 months. Kelley (15) found that after 90 days approximately 20 % of the protein was water-soluble. The comparative figure given graphically by Sherwood (16,17) for his control Cheddar cheese was 31·0 %. Thus the corresponding value given in the present study for cows' milk Cheddar cheese is

Table 3. *Cheese yield*

Milk	Cheddar	Derby	Manfis
Cows	8·39	9·12	8·61
Buffaloes	12·24	12·72	12·71

Table 4. *Average composition of cheese*

	Cheddar		Derby		Manfis	
	Cows	Buffaloes	Cows	Buffaloes	Cows	Buffaloes
Moisture (%)	32·25	28·42	38·29	30·80	36·77	32·70
Fat (%)	34·96	41·96	30·31	40·04	31·51	39·27
Total N (%)	3·94	3·60	3·78	3·45	3·90	3·32
Soluble N (%)	0·953	0·622	0·680	0·509	1·093	0·728
Non-protein N (%)	0·813	0·520	0·568	0·398	0·849	0·564
Salt (%)	1·67	1·64	1·83	1·75	2·47	2·23
Acidity (%)	2·97	2·51	2·62	2·23	2·15	1·90
Soluble N % of total N	24·20	17·29	18·05	14·75	28·12	22·07
Non-protein N % of total N	20·66	14·45	15·09	11·55	21·77	17·07

within the limits stated by these investigators, although the value shown by Sherwood for the non-protein N as a percentage of the total N, namely, 25 %, after 12 weeks is higher than the corresponding value obtained by us.

Cheese made from the two kinds of milk differ considerably in composition. The differences (Table 5) are consistently in the same direction with all types of cheese. The results of the ten experiments have, therefore, been combined for cheese made from each kind of milk, to calculate the significance of the differences shown in Table 5. The mean values of moisture, total N, soluble N, non-protein N, salt and acidity are higher in cows' milk cheese than in those of buffaloes' by 5·02, 0·43, 0·297, 0·253, 0·13 and 0·36 % respectively, while the fat content of the latter is 8·13 % higher than that of the former. The soluble N and non-protein N contents expressed as percentages of the total N (Table 6) are higher for cows' than for buffaloes' milk cheese, the differences being 5·48 and 4·80 % respectively.

In spite of the wide variation in the composition of the different types of cheese, the differences between cows' and buffaloes' milk cheese are all significant, except with the salt content. The differences between cheese of both milks within each type may, therefore, be accepted as highly significant.

Table 5. *Difference in composition between cows' and buffaloes' ripened cheese*

	Moisture (%)		Fat (%)		Total N (%)		Soluble N (%)		Non-protein N (%)		Salt (%)		Acidity (%)	
	C	*B*	*C*	*B*	*C*	*B*	*C*	*B*	*C*	*B*	*C*	*B*	*C*	*B*
Mean	35·87	30·85	32·18	40·31	3·88	3·45	0·927	0·630	0·754	0·501	2·04	1·91	2·54	2·18
Range of variation	30·40–40·74	26·22–35·90	29·12–37·14	36·82–43·94	3·58–4·17	3·05–3·78	0·654–1·172	0·489–0·762	0·552–0·937	0·376–0·600	1·42–2·78	1·44–2·88	2·03–3·09	1·71–2·92
S.D.	2·98	2·96	2·51	2·50	0·22	0·22	0·190	0·100	0·143	0·077	0·44	0·45	0·40	0·36
C.V.	0·08	0·10	0·08	0·06	0·06	0·06	0·205	0·159	0·190	0·154	0·22	0·24	0·16	0·17
S.E.M.	0·94	0·94	0·79	0·79	0·07	0·07	0·060	0·032	0·045	0·024	0·14	0·14	0·13	0·11
Diff. between the two means *C–B*	+5·02		−8·13		+0·43		+0·297		+0·253		+0·13		+0·36	
T	3·780		7·265		4·450		4·370		4·918		0·653		2·118	
P	<0·01		<0·01		<0·01		<0·01		<0·01		0·5–0·6		0·02–0·05	
Sig.	+		+		+		+		+		−		+	

C = cows. *B* = buffaloes. + = significant. − = insignificant.

Table 6. *Difference between the average soluble* N *and non-protein* N *in buffaloes' and cows' ripened cheese (calculated as percentage of total* N)

	Soluble N (%)		Non-protein N (%)	
	C	*B*	*C*	*B*
Mean	23·92	18·44	19·43	14·63
Range of variation	17·02–30·53	14·25–25·00	14·04–23·63	10·97–18·52
S.D.	4·86	3·72	3·47	2·69
C.V.	0·20	0·20	0·18	0·18
S.E.M.	1·54	1·18	1·10	0·85
Difference between two means *C–B*	+5·48		+4·80	
T	2·834		3·462	
P	0·01–0·02		<0·01	
Sig.	+		+	

C = cows. *B* = buffaloes. + = significant.

Cheese quality

It is clear that cows' milk cheese differs considerably from that of buffaloes'. The latter is characterized by a significantly lower moisture, protein and acidity content, together with a higher percentage of fat. It is also of much lower quality. Table 7 shows the average score-points allowed for each type of cheese as obtained from both kinds of milk.

On combining the scores for the ten experiments (Table 8) cows' milk cheese obtained a significantly higher score than that of buffaloes'.

There is lack of agreement among practical authorities and scientific investigators concerning the relationship between the composition and the quality of cheese. Comparing cheese made from the milks of different breeds of cows, it has been shown that apparently the richest milk makes the best cheese (6). Recent observations seem to indicate that,

Table 7. *Average score points for each type of cheese*

	Flavour and aroma		Body and texture		Total	
	Cows	Buffaloes	Cows	Buffaloes	Cows	Buffaloes
Cheddar	51	43	36	29	87	72
Derby	51	47	35	30	86	77
Manfis	49	46	33	25	82	71

Table 8. *Difference between the average score points for cows' and buffaloes' cheese*

(Ten experiments)

	Flavour and aroma 60		Body and texture 40		Total score 100	
	C	*B*	*C*	*B*	*C*	*B*
Mean	50	45	35	28	85	73
Range of variation	40–56	36–53	27–37	21–33	67–93	64–84
S.D.	4·56	5·99	3·11	4·09	7·33	7·47
C.V.	0·09	0·13	0·09	0·15	0·09	0·10
S.E.M.	1·44	1·90	0·98	1·30	2·32	2·37
Difference between the two means *C*–*B*	+5·0		+7·0		+12·0	
T	2·101		4·305		3·630	
P	0·05		<0·01		0·01	
Sig.	+		+		+	

C = cows. *B* = buffaloes. + = significant.

with the normal range of fat content, it is difficult to distinguish between cheese produced from low-fat and high-fat milks. Although some investigators found that larger proportions of fat in cheese tended to be associated with slightly higher scores, yet the correlation was not significant, nor was it commercially important. On the other hand, Price & North (18), investigating the mechanical control of fat in Swiss cheese-making, came to the conclusion that the best grades were associated with less fat in the dry matter of cheese.

Many investigators have also studied the effect of acidity on the quality of cheese, and concluded that it is an important factor influencing the nature of cheese (6,19,20,21). Others have shown the decided influence of moisture (6,14,22,23). The inferior quality of buffaloes' milk hard cheese, in spite of its higher fat content, is not, therefore, unexpected owing to its lower acidity and moisture contents associated with less extensive protein degradation.

Section II

The previous observations concerning the composition of both cows' and buffaloes' milks and wheys, together with their corresponding cheese-making characteristics,

discussed in § I, were confirmed by the results of this section (Table 2). It must, however, be mentioned that a much longer period was given to buffaloes' milk curd than that given to cows', until both curds produced the same length of threads on the hot iron. This is clearly seen from the results of the second set.

Table 9. *Average composition of cheese*

	Moisture (%)		Fat (%)		Total N (%)		Soluble N (%)		Non-protein N (%)		Salt (%)		Acidity (%)	
Intervals	*C*	*B*	*C*	*B*	*C*	*B*	*C*	*B*	*C*	*B*	*C*	*B*	*C*	*B*
First set (Exps. no. 1–5)														
1 day	45·95	40·59	26·63	33·94	3·35	2·98	0·164	0·117	0·119	0·099	1·86	1·84	1·35	0·82
1 week	45·05	38·96	—	—	3·40	3·08	0·296	0·194	0·198	0·136	—	—	1·84	0·99
2 weeks	44·24	37·66	—	—	3·44	3·15	0·408	0·236	0·263	0·161	—	—	2·45	1·47
4 weeks	42·90	35·47	—	—	3·51	3·24	0·546	0·328	0·374	0·230	—	—	2·64	1·73
6 weeks	41·71	33·99	—	—	3·58	3·30	0·635	0·395	0·511	0·299	—	—	2·70	1·82
8 weeks	40·05	32·62	29·62	38·63	3·71	3·38	0·734	0·488	0·626	0·397	2·09	2·09	2·79	1·93
Second set (Exps. nos. 6–10)														
1 day	46·33	39·07	26·80	34·43	3·22	3·14	0·147	0·135	0·085	0·081	1·78	1·64	1·25	1·19
1 week	45·62	38·17	—	—	3·27	3·19	0·264	0·190	0·177	0·148	—	—	1·75	1·54
2 weeks	44·75	37·61	—	—	3·30	3·22	0·321	0·274	0·233	0·206	—	—	2·13	1·90
4 weeks	43·85	36·49	—	—	3·36	3·28	0·472	0·436	0·365	0·312	—	—	2·45	2·25
6 weeks	42·85	35·28	—	—	3·42	3·35	0·609	0·519	0·467	0·381	—	—	2·64	2·36
8 weeks	42·15	34·11	28·78	37·44	3·47	3·41	0·723	0·589	0·597	0·469	1·97	1·81	2·80	2·49

C = cows. *B* = buffaloes.

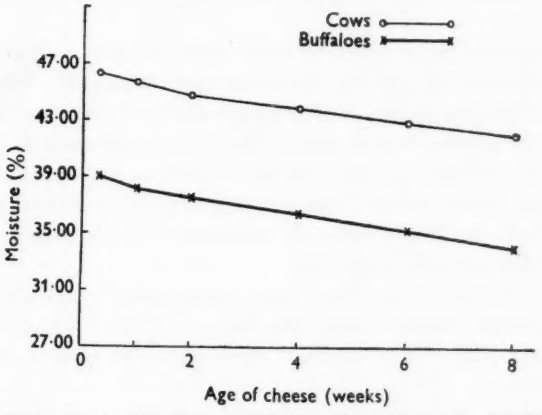

Fig. 1. 2nd set (Exps. 6–10). Average rate of the loss of moisture in cows' and buffaloes' milk cheeses during ripening.

The results of the first set of experiments (nos. 1–5) show that the moisture content of cows' green cheese averaged 45·95 %, falling at the end of 8 weeks to 40·05 %, thus losing 5·90 % during the ripening period (Table 9). In buffaloes' milk cheese this loss is greater in spite of the lower moisture content of the green cheese. The average value in this case falls from 40·59 to 32·62 %, with an average loss of 7·97 %. With the second set (Table 9 and Fig. 1) this loss was again greater with buffaloes' milk cheese. The difference in this case, however, is much smaller than with the first set; the two respective losses with buffaloes' and cows' milk cheese being 4·96 and 4·18 %. Buffaloes' milk cheese made in the second set lost, on the average, less moisture than that made in the first. This may be explained by the lower moisture content of the green cheese in the former set, lower

temperature of ripening or higher acidity at the beginning of ripening as referred to by Van Slyke (6).

The results show that the capacity of buffaloes' milk curd to hold moisture is much lower than that of cows', due no doubt to the higher casein and fat content of buffaloes' milk. Sanders, Matheson & Burkey (24) stated that the percentage of casein in milk exerted a considerable influence on the firmness of curd, *i.e.* the more the casein the firmer the curd and the less moisture it retains. Also Sanders & Farrar (25) showed that, as the milk fat content increased, the percentage of moisture in the green cheese decreased. These findings have been confirmed by other investigators (23,26).

Sammis & Germain (27) showed that, in Cheddar cheese, ripening is influenced by the ratio of moisture/solids-not-fat, and pointed out that bacterial growth and chemical changes are more rapid in cheese of high moisture content. Thus, comparing the rate of acid development in buffaloes' and cows' milk cheese, in both sets of experiments, this rate is slower with the former than with the latter, but comparison of the rate in buffalo cheese of both sets shows that it is faster in the second set owing to the higher acidity at milling. According to Spicer & Burgwald (28) acid development in cheese during storage is closely related to the titratable acidity of the curd at the time of milling (30). Although cows' curd has a shorter Cheddaring period, it always produces green cheese of noticeably higher acidity than buffaloes'. The difference between the two kinds is, however, more pronounced in the first than in the second set, owing to the higher milling acidity in the latter.

Table 9 also shows that the green cheese made from buffaloes' milk contain much lower average total N and higher fat contents than those made from cows'. This is obviously due to the variation in the composition of the original milks. Protein degradation, measured as soluble N and non-protein N, and also by these values expressed as percentages of the total N, is higher in the first set of green cheese made from cows' milk than in those made from buffaloes'. In the second set, however, these values are remarkably close in both kinds of cheese (Table 9). This is evidently associated with the higher milling acidity of buffaloes' curd in this set than in the first.

The rate of protein degradation then shows a progressive increase as the cheese ages. The maximum increase occurs during the first week, after which it continues at a uniform but slower rate. These findings agree with the results of several investigators (14,16,17,29,30,31).

The rate of protein breakdown is slower in buffaloes' than in cows' milk cheese, particularly in the first set. The reason for this difference appears to lie in the wide variation in acid development which occurred during the manufacture of cheese from both kinds of milks, and also in the moisture content retained in the cheese.

Composition of mature cheese

The statistical significance of differences between the composition of mature cheese is given in Tables 10–12. In the first set, the average values of moisture, total N, soluble N, non-protein N and acidity content are significantly higher for cows' milk cheese, while the fat content is lower. In the second set, cows' milk cheese again contains significantly more moisture and acidity, and less fat than buffaloes', but total N, soluble N, non-protein N and salt contents are only insignificantly higher. Expressing the values of soluble and non-protein N contents as percentages of total N, Table 12 shows that, with the first set,

Table 10. *Difference between the composition of cows' and buffaloes' cheese*

	Moisture (%)		Fat (%)		Total N (%)		Soluble N (%)		Non-protein N (%)		Salt (%)		Acidity (%)	
	C	*B*	*C*	*B*	*C*	*B*	*C*	*B*	*C*	*B*	*C*	*B*	*C*	*B*
First set (Exps. nos. 1–5)														
Mean	40·05	32·62	29·62	38·63	3·71	3·38	0·734	0·488	0·626	0·397	2·09	2·09	2·79	1·93
Range of variation	38·13–42·52	29·69–37·64	28·03–30·56	34·17–41·20	3·50–3·85	3·15–3·61	0·650–0·855	0·399–0·618	0·552–0·722	0·330–0·494	1·98–2·27	1·82–2·39	2·58–3·05	1·74–2·17
S.D.	1·59	3·53	1·10	2·69	0·13	0·17	0·078	0·087	0·074	0·070	0·12	0·27	0·18	0·16
C.V.	0·04	0·11	0·04	0·07	0·03	0·05	0·106	0·178	0·118	0·176	0·06	0·13	0·06	0·08
S.E.M.	0·71	1·58	0·49	1·20	0·06	0·08	0·035	0·039	0·033	0·031	0·05	0·12	0·08	0·07
Diff. between the two means *C–B*	+7·43		−9·01		+0·33		+0·246		+0·229		0·00		+0·86	
T	4·295		6·950		3·425		4·700		5·047		0·000		8·020	
P	<0·01		<0·01		<0·01		<0·01		<0·01		—		<0·01	
Sig.	+		+		+		+		+		—		+	
Second set (Exps. nos. 6–10)														
Mean	42·15	34·11	28·78	37·44	3·47	3·41	0·723	0·589	0·597	0·469	1·97	1·81	2·80	2·49
Range of variation	39·63–45·00	30·97–37·59	26·97–30·59	35·00–40·37	3·35–3·58	3·21–3·54	0·581–0·851	0·506–0·675	0·514–0·672	0·413–0·563	1·83–2·15	1·56–2·20	2·67–3·18	2·24–2·73
S.D.	2·27	2·99	1·49	2·29	0·10	0·16	0·127	0·075	0·078	0·066	0·15	0·24	0·21	0·20
C.V.	0·05	0·09	0·05	0·06	0·03	0·05	0·176	0·127	0·131	0·141	0·08	0·13	0·08	0·08
S.E.M.	1·02	1·34	0·67	1·02	0·05	0·07	0·057	0·034	0·035	0·030	0·07	0·11	0·09	0·09
Diff. between the two means *C–B*	+8·04		−8·66		+0·06		+0·134		+0·128		+0·16		+0·31	
T	4·788		7·082		0·707		2·040		2·792		1·279		2·348	
P	<0·01		<0·01		0·4–0·5		0·05–0·1		0·02–0·05		0·2–0·3		0·02–0·05	
Sig.	+		+		−		−		+		−		+	

C = cows. *B* = buffaloes. + = significant.

Table 11. *Average soluble* N *and non-protein* N *in cows' and buffaloes' cheese at intervals calculated as percentages of total* N (*the two sets*)

	First set				Second set			
	Soluble N (%)		Non-protein N (%)		Soluble N (%)		Non-protein N (%)	
Intervals	*C*	*B*	*C*	*B*	*C*	*B*	*C*	*B*
1 day	4·90	3·94	3·54	3·32	4·57	4·30	2·64	2·59
1 week	8·68	6·33	5·82	4·42	8·10	5·95	5·42	4·64
2 weeks	11·83	7·49	7·61	5·11	9·76	8·51	7·06	6·38
4 weeks	15·48	10·13	10·63	7·10	14·09	13·28	10·90	9·52
6 weeks	17·70	12·01	14·27	9·08	17·85	15·52	13·68	11·41
8 weeks	19·80	14·46	16·85	11·76	20·90	17·31	17·23	13·80

C = cows. *B* = buffaloes.

Table 12. *Difference between the average soluble N and non-protein* N *in cows' and buffaloes' cheese. Calculated as percentages of total* N (*the two sets*)

	First set				Second set			
	Soluble N (%)		Non-protein N (%)		Soluble N (%)		Non-protein N (%)	
	C	*B*	*C*	*B*	*C*	*B*	*C*	*B*
Mean	19·80	14·46	16·85	11·76	20·90	17·31	17·23	13·80
Range of variation	17·44–22·65	11·68–18·44	15·03–19·10	9·36–14·76	16·23–25·42	15·63–20·70	14·42–20·02	12·08–17·28
S.D.	1·89	2·78	1·59	2·19	3·99	2·34	2·52	2·37
C.V.	0·10	0·19	0·09	0·19	0·19	0·14	0·15	0·17
S.E.M.	0·85	1·24	0·71	0·98	1·78	1·05	1·13	1·06
Diff. between the two means *C*—*B*	+5·34		+5·09		+3·59		+3·43	
T	3·555		4·200		1·736		2·218	
P	<0·01		<0·01		0·1–0·2		0·05–0·1	
Sig.	+		+		−		−	

+ = significant. *C* = cows. *B* = buffaloes.

Table 13. *Difference between the composition of buffaloes' cheese*

	Moisture (%)		Fat (%)		Total N (%)		Soluble N (%)		Non-protein N (%)		Salt (%)		Acidity (%)	
	First set	Second set	First set	Second set	First set	Second set	First set	Second set	First set	Second set	First set	Second set	First set	Second set
Mean	32·62	34·11	38·63	37·44	3·38	3·41	0·488	0·589	0·397	0·469	2·09	1·81	1·93	2·49
Range of variation	29·69–37·64	30·97–37·59	34·17–41·20	35·00–40·37	3·15–3·61	3·21–3·54	0·399–0·618	0·506–0·675	0·330–0·494	0·413–0·563	1·82–2·39	1·56–2·20	1·74–2·17	2·24–2·73
S.D.	3·53	2·99	2·69	2·29	0·17	0·16	0·087	0·075	0·070	0·066	0·27	0·24	0·16	0·20
C.V.	0·11	0·09	0·07	0·06	0·05	0·05	0·178	0·127	0·176	0·141	0·13	0·13	0·08	0·08
S.E.M.	1·58	1·34	1·20	1·02	0·08	0·07	0·039	0·034	0·031	0·030	0·12	0·11	0·07	0·09
Diff. between the two means B_1–B	+1·49		−1·19		+0·03		+0·101		+0·072		−0·28		+0·56	
T	0·720		0·753		0·286		1·967		1·678		1·738		4·867	
P	0·4–0·5		0·4–0·5		0·7–0·8		0·05–0·1		0·1–0·2		0·1–0·2		<0·01	
Sig.	−		−		−		−		−		−		+	

+ =significant. − =insignificant.

Table 14. *Difference between the average soluble* N *and non-protein* N *in buffaloes' ripened cheese of the two sets calculated as percentages of total* N

	Soluble N (%)		Non-protein N (%)	
	First set	Second set	First set	Second set
Mean	14·46	17·31	11·76	13·80
Range of variation	11·68–18·44	15·63–20·70	9·36–14·76	12·08–17·28
S.D.	2·78	2·34	2·19	2·37
C.V.	0·19	0·14	0·19	0·17
S.E.M.	1·24	1·05	0·98	1·06
Diff. between the two means B_1–B	+2·85		+2·04	
T	1·758		1·414	
P	0·1–0·2		0·1–0·2	
Sig.	−		−	

+ =significant. − =insignificant.

these values are significantly higher in cows' milk cheese than those of buffaloes'. In the second set they are again higher, but the differences are statistically insignificant.

Comparing buffaloes' milk cheese of both sets, Tables 13 and 14 show that the most distinct differences lie in the values of moisture, soluble N, non-protein N and acidity; all being higher for the second set than the first by 1·49, 0·101, 0·072 and 0·56 % respectively. The soluble N and non-protein N values expressed as percentages of the total N are also higher for the first set by 2·85 and 2·04 % respectively. All differences except acidity are, however, not significant.

These observations show the important part played by acidity in ripening, and may explain the smaller differences between cows' and buffaloes' milk cheese in the second set.

Table 15. *Difference between the average score points given for cows' and buffaloes' ripened cheese (the two sets)*

	First set						Second set					
	Flavour and aroma 60		Body and texture 40		Total 100		Flavour and aroma 60		Body and texture 40		Total 100	
	C	*B*	*C*	*B*	*C*	*B*	*C*	*B*	*C*	*B*	*C*	*B*
Mean	50	35	35	25	35	60	51	41	34	29	85	70
Range of variation	40–53	27–47	33–37	13–35	73–89	40–82	47–56	33–47	32–37	23–33	80–91	62–80
S.D.	5·48	8·63	1·58	8·50	6·82	15·95	4·12	5·87	2·00	3·87	5·15	8·46
C.V.	0·11	0·25	0·05	0·34	0·08	0·27	0·08	0·14	0·06	0·13	0·06	0·12
S.E.M.	2·45	3·86	0·71	3·80	3·05	7·13	1·84	2·63	0·89	1·73	2·30	3·78
Diff. between the two means *C*–*B*	+15		+10		+25		+10		+5		+15	
T	3·282		2·585		3·225		3·125		2·561		3·384	
P	0·01–0·02		0·02–0·05		0·01–0·02		0·01–0·02		0·02–0·05		<0·01	
Sig.	+		+		+		+		+		+	

C = cows. *B* = buffaloes. + = significant.

Table 16. *Difference between the average score points given for buffaloes' ripened cheese in both sets*

	Flavour and aroma 60		Body and texture 40		Total 100	
	First set *B*	Second set B_1	First set *B*	Second set B_1	First set *B*	Second set B_1
Mean	35	41	25	29	60	70
Range of variation	27–47	33–47	13–35	23–33	40–82	62–80
S.D.	8·63	5·87	8·50	3·87	15·95	8·46
C.V.	0·25	0·14	0·34	0·13	0·27	0·12
S.E.M.	3·86	2·63	3·80	1·73	7·13	3·78
Diff. between the two means B_1–*B*	+6		+4		+10	
T	1·287		0·957		1·240	
P	0·2–0·3		0·3–0·4		0·2–0·3	
Sig.	–		–		–	

+ = significant. – = insignificant.

Cheese quality

As in § I, buffaloes' milk cheese are of significantly lower score than those of cows' (Table 15). On the other hand, Table 16 shows that, although buffaloes' milk cheese obtained a lower score in the first set of experiments than in the second, the differences are not significant. Therefore, leaving buffaloes' curd for a longer time in Cheddaring with the second set of experiments has apparently raised the milling acidity and exerted some effect in improving the quality of cheese.

ATTEMPTS TO IMPROVE THE QUALITY OF BUFFALOES' MILK CHEESE

Attempts to improve the quality of buffalo cheese must aim at minimizing the differences between it and cows' milk cheese. In 1941 Hassouna tried the effect of adding water to buffaloes' milk (32). But, since curds containing additional water seemed, as found by Samis (33), to lose their extra moisture rapidly in the cheese vat, their water/solids-not-fat finally reaching the ordinary level, further investigation has been undertaken to test other modifications. The enzymatic breakdown of the caseins in both milks was studied by one of us, and it was concluded that the differences in their rate of proteolysis could not be related to differences in their nature.

Since the extent of protein degradation in cheese is a major factor in the development of its flavour, aroma, body and texture, the problem of improving buffaloes' milk hard cheese seems to lie, mainly, in attaining the proper conditions for proteolysis. The previous experiments have shown that this is governed, to a large extent, by the moisture content of the cheese, and also by the rate of acid development during the process of manufacture and the period of ripening, particularly in the early stages when the rennet enzymes are most active (17).

Experiments on Derby cheese-making from buffaloes' milk were carried out to study the effect of acidity, modification of the composition of milk and modifications in the process of manufacture. The experiments may be summarized as follows:

(*a*) Prolonged ripening of buffaloes' milk to an acidity of 0·21–0·22 %.

(*b*) Modification of the casein/fat ratio in this milk to approximately the same ratio as in cows' milk.

(*c*) Modification of the above ratio together with prolonged ripening of the milk.

(*d*) Modification of the ratio plus low scalding temperature of the curd, the time of scalding being reduced from 60 to 30 min.

Determinations were made of fat and acidity in milk and whey, and of moisture, total N, soluble N, non-protein N and acidity in green cheese, after removal from the press, and at intervals of 1, 2, 4, 6 and 8 weeks during ripening. Fat and salt content were determined only in green and mature cheese.

METHODS OF ANALYSIS

The methods used were the same as before with the addition of the formol titration test carried out as follows: Ten ml. of milk were titrated with N/10-sodium hydroxide, using phenolphthalein as indicator. Then 2 ml. of 40 % formaldehyde solution were added and the mixture again neutralized with the same solution of sodium hydroxide. The blank was determined and deducted from the amount of the second volume of alkali used. Modified figures for casein N were used for the calculation, 0·2118 per 1 ml. of alkali for cows' milk and 0·2166 for buffaloes'. The results were multiplied by the factor 6·38 to give the casein percentage in milk. The casein/fat ratio of the buffaloes' milk was modified on the basis of the results of the above titration (the accuracy was checked by Kjeldahl determinations made later) and on that of the fat estimation by the Gerber method. The amount of separated milk necessary to bring the ratio to the same as that in the control cows' milk of the same experiment was calculated and added. Both whole and separated buffaloes' milk were taken from the same batch.

RESULTS AND DISCUSSION

(*a*) *Effect of prolonged ripening of milk*

Three experiments were undertaken in which starter was added to a bulk of 120 lb. of buffaloes' milk which was thoroughly mixed and divided into two equal parts, each being strained in a separate vat. One part was renneted at an acidity of 0·19 %, while the other was left until the acidity reached 0·21 %. The procedure was then continued according to the general method for Derby cheese. The typical manufacturing details were similar with both batches of milk in the three experiments, as follows:

Quantity of milk (lb.)	60
Quantity of starter (lb.)	$\frac{1}{6}$
Renneting temperature (° F.)	85
Scalding temperature (° F.)	95
Time of scalding (min.)	60
Acidity whey drawn off (%)	0·145
Acidity at milling (inch of hot iron)	$\frac{1}{2}$

Table 17. *Variable steps of manufacture*

	First experiment		Second experiment		Third experiment	
	Control	Experimental	Control	Experimental	Control	Experimental
Fat in milk (%)	5·30	5·30	5·35	5·35	5·30	5·30
Renneting acidity (%)	0·190	0·210	0·190	0·210	0·190	0·210
Time of milk ripening (min.)	30	210	40	225	30	180
Acidity cutting (%)	0·115	0·135	0·110	0·125	0·110	0·135
Time of pitching (min.)	125	5	170	15	105	5
Time of Cheddaring (min.)	140	110	120	120	110	120

Table 18. *Average composition of cheese at intervals*

(First series, Exps. nos. 1–3)

	Moisture (%)		Fat (%)		Total N (%)		Soluble N (%)		Non-protein N (%)		Salt (%)		Acidity (%)	
Intervals	*B*	B_1	*B*	B_1	*B*	B_1	*B*	B_1	*B*	B_1	*B*	B_1	*B*	B_1
1 day	37·30	36·98	34·61	34·32	3·51	3·60	0·143	0·163	0·096	0·111	1·31	1·21	1·57	1·62
1 week	35·04	35·02	—	—	3·65	3·72	0·279	0·334	0·191	0·242	—	—	2·18	2·23
2 weeks	33·66	33·58	—	—	3·71	3·79	0·354	0·435	0·265	0·327	—	—	2·29	2·40
4 weeks	31·53	31·21	—	—	3·84	3·93	0·439	0·531	0·333	0·403	—	—	2·36	2·51
6 weeks	30·35	30·10	—	—	3·90	3·98	0·486	0·587	0·367	0·459	—	—	2·43	2·57
8 weeks	29·26	28·85	39·12	38·98	3·94	4·04	0·524	0·620	0·441	0·508	1·48	1·39	2·44	2·58

Other data are given in Table 17, from which it will be observed that the prolonged ripening of buffaloes' milk to 0·21 % acidity considerably shortened the pitching step; the average difference between the two batches in the three experiments was 125 min. On the other hand, the total time required for the whole cheese-making process averaged 30 min. longer for the ripened vat than for the control.

Table 18 shows that the moisture content of green cheese is slightly higher in the contro than in the experimental cheese, the two averages being 37·30 and 36·98 % respectively. Subsequent loss of moisture was highest at the end of the first week. The two values run closely parallel, giving almost identical averages at the end of ripening, namely, 29·26 % in the control and 28·85 % in the experimental cheese. Compared with the average moisture content of cows' milk cheese these values are very low. Hence, high acidities at

renneting or milling, for buffaloes' milk, cause excessive loss of moisture from the curd and produce very dry cheese. This agrees with the results of previous workers on cows' milk[34,35,25,6,7].

The average rate of acid development in cheese during ripening (Table 18) shows a peak at the end of the first week. This is in harmony with the results of several investigators[36,37,38], but it differs slightly from our previous results, which showed that this peak occurred within 2 weeks. This difference is attributed to the higher milling acidity attained in the present experiments. Comparing the control with the experimental cheese, it will also be observed that there is very little difference between the rates of acid development in both cheese. The final average values in mature cheese are 2·44 and 2·58 % respectively. These values are higher than our earlier figures for buffalo cheese, suggesting that the milling acidity influences the rate of acid development during ripening more than does the renneting acidity.

These higher acidities influence the rate of protein breakdown, since the values of soluble nitrogen and non-protein nitrogen in green cheese (Table 18) approach, or may even exceed, those obtained with cows' milk cheese. This effect continued for some time during ripening, but after about 4 weeks the rate declined, influenced by the very low moisture content. Van Slyke & Price[6] consider that below 26 % moisture content no proteolytic action can occur. The average moisture content of both cheese approach this value as the rate of proteolysis gets slower (Table 18). Consequently, the final average soluble and non-protein nitrogen contents at the end of the ripening period are distinctly lower than the corresponding values usually observed with cows' milk cheese.

Both cheese were considered to be of a low quality, with a lower score for the experimental than for the control cheese, as follows:

Average score

	Flavour and aroma	Body and texture	Total
Control	41	27	68
Experimental	38	29	67

The lower average score for flavour and aroma in the case of the experimental cheese is due to the development of taints in two out of three experiments.

Thus, although the prolonged ripening of buffaloes' milk before renneting causes the acidity of whey at cutting to approach the relative value of cows', and also shortens the pitching step considerably, it gives cheese of much lower moisture contents than those of cows' milk. The effects of this low moisture content, which slows down the rate of proteolysis at the late stages of cheese ripening, with the possibility of taint development during the ripening of milk, suggest that prolonged ripening of the curd might be preferable to prolonged ripening of milk.

(*b*) *Effect of modifying the casein/fat ratio*

Three experiments were made to investigate the effect of this modification. In each experiment three batches of milk were taken. One of these batches was from cows' herd milk for comparison, the second was from that of buffaloes as control, and the third from the same batch of buffaloes' milk with its casein/fat ratio modified to the same ratio as in the batch of cows' milk in the same experiment. The procedure of manufacture was the same as that described for the control in the previous series of experiments, with the

exception of some significant differences shown in Table 19. The quantity of cows' milk in each experiment was 80 lb., while that of both batches of buffaloes' milk was only 60 lb.

Even after modification of the casein/fat ratio, buffaloes' milk still requires longer time to make than cows'. There is no appreciable difference between the two batches of buffaloes' milk in this respect (Table 19). This agrees with the findings of Sanders, Farrar, Hardell, Feutz & Burkey(26). The rate of loss of moisture during ripening is slightly greater for the modified buffaloes' milk cheese than for the control, but the former still has a higher final moisture content. Comparison shows that cows' milk cheese contains much more moisture than the other two, both at the beginning and end of ripening.

Table 19. *Cheese-making data*

	Experiment 1			Experiment 2			Experiment 3		
	C	*B*	B_1	*C*	*B*	B_1	*C*	*B*	B_1
Acidity of milk (%)	0·200	0·175	0·175	0·200	0·180	0·180	0·185	0·185	0·185
Fat in milk (%)	3·60	5·20	4·20	3·50	5·25	4·30	3·40	5·00	4·25
Casein/fat ratio	0·75	0·62	0·77	0·72	0·61	0·74	0·74	0·64	0·75
Acidity at renneting (%)	0·200	0·190	0·190	0·200	0·190	0·190	0·190	0·190	0·190
Time of ripening (min.)	20	80	80	20	75	75	40	80	80
Acidity at cutting (%)	0·135	0·105	0·105	0·140	0·110	0·110	0·135	0·115	0·110
Time of scalding (min.)	50	60	60	50	60	60	60	60	60
Time of pitching (min.)	5	160	175	5	55	60	5	90	95
Acidity whey drawn off (%)	0·150	0·145	0·145	0·150	0·145	0·145	0·145	0·145	0·145
Time of Cheddaring (min.)	110	145	145	110	155	150	130	180	175

C = cows. *B* = buffaloes' control. B_1 = modified buffaloes' experimental.

Table 20. *Average composition of cheese at intervals*

(Second series, Exps. nos. 4–6)

	Moisture (%)			Fat (%)			Total N (%)			Soluble N (%)			Non-protein N (%)			Salt (%)			Acidity (%)		
Intervals	*C*	*B*	B_1	*C*	*B*	B_1	*C*	*B*	B_1	*C*	*B*	B_1	*C*	*B*	B_1	*C*	*B*	B_1	*C*	*B*	B_1
1 day	48·23	38·21	40·12	26·02	33·09	30·20	3·14	3·54	3·73	0·125	0·133	0·141	0·072	0·085	0·094	1·48	1·37	1·34	1·22	1·45	1·53
1 week	47·14	37·14	39·15	—	—	—	3·20	3·59	3·79	0·200	0·231	0·238	0·125	0·152	0·173	—	—	—	1·70	1·87	2·01
2 weeks	46·42	36·79	38·44	—	—	—	3·24	3·61	3·82	0·282	0·275	0·310	0·180	0·196	0·214	—	—	—	2·02	2·03	2·14
4 weeks	45·46	35·94	37·38	—	—	—	3·29	3·68	3·89	0·432	0·417	0·455	0·307	0·285	0·312	—	—	—	2·23	2·23	2·37
6 weeks	44·39	34·63	36·31	—	—	—	3·37	3·75	3·95	0·592	0·506	0·576	0·414	0·366	0·415	—	—	—	2·30	2·35	2·44
8 weeks	42·83	33·45	35·00	29·06	35·81	33·20	3·45	3·81	4·05	0·743	0·576	0·666	0·546	0·424	0·495	1·66	1·51	1·47	2·38	2·44	2·56

C = cows. *B* = control buffaloes. B_1 = modified buffaloes.

Considering the rate of acid development, it will be observed (Table 20) that the two average values for both the control and experimental buffaloes' milk cheese when mature are slightly higher than that of cows' at the same stage.

Moreover, coinciding with the beneficial effect of the modification on the moisture content, the rate of protein degradation is (Table 20) much faster in the modified buffaloes' milk cheese than in the control. This, however, is more marked during the advanced stage of ripening. During the first 4 weeks the rate is lower for cows' milk cheese than for the experimental buffaloes', and lower than the control buffaloes for the first 2 weeks of ripening. Subsequently, however, ripening proceeded more rapidly in the cheese made from cows' milk, causing its soluble and non-protein-nitrogen contents to be much higher than those of the other two cheese.

The effect of modification on the final quality of cheese is given below. Although the average total score awarded to the modified buffaloes' milk cheese is even higher than that awarded to cows' milk cheese, the average flavour score is higher for the latter than for both cheese made from buffaloes' milk. This indicates that cows' milk cheese is still of

better flavour and aroma; this agrees with the results of the chemical analysis. Its lower score for body and texture is due to an unexpectedly higher moisture content; this was avoided in the next series.

Average score

	Flavour and aroma	Body and texture	Total
Cows'	47	25	72
Buffaloes' control	38	29	67
Buffaloes' experimental	42	33	75

In general, the modification yielded some improvement, but more work was still needed to attain the standard of cows' milk cheese.

(*c*) *Effect of ripening buffaloes' milk and modifying its casein/fat ratio*

In this series of three experiments, the experimental buffaloes' milk cheese were made from milk in which the casein/fat ratio was first modified as in the previous series (*b*); the milk was then ripened to an acidity of 0·21 % as in the first series (*a*).

Table 21 shows the manufacturing data which varied from one batch to another.

Table 21. *Cheese-making data*

	Experiment 1			Experiment 2			Experiment 3		
	C	*B*	B_1	*C*	*B*	B_1	*C*	*B*	B_1
Acidity of milk (%)	0·205	0·185	0·185	0·200	0·185	0·185	0·200	0·170	0·170
Fat in milk (%)	3·75	5·05	4·40	3·00	5·45	3·80	3·20	5·40	4·20
Amount of starter (lb.)	¼	⅛	⅛	¼	⅛	⅛	¼	⅛	⅛
Casein/fat ratio	0·69	0·57	0·66	0·80	0·56	0·83	0·76	0·59	0·76
Acidity at renneting (%)	0·210	0·190	0·210	0·200	0·190	0·210	0·205	0·190	0·210
Time of ripening (min.)	30	35	255	35	55	165	35	180	260
Acidity at cutting (%)	0·135	0·110	0·135	0·135	0·110	0·125	0·130	0·110	0·140
Time of scalding (min.)	50	60	60	50	60	60	60	60	60
Time of pitching (min.)	5	100	5	5	115	55	5	55	5
Acidity whey drawn off (%)	0·145	0·145	0·150	0·145	0·145	0·145	0·145	0·145	0·150
Time of Cheddaring (min.)	110	180	115	100	150	160	130	145	120

C = cows. *B* = buffaloes' control. B_1 = buffaloes' experimental.

The results of this series of experiments (Table 22) confirm those obtained in the first series with regard to the effect of the prolonged ripening of buffaloes' milk on the composition, ripening and quality of the resultant cheese. The effects of modifying the casein/fat ratio in the same kind of milk also confirm the results obtained in the second series. The average cheese scores, given below, show that, although buffaloes' milk experimental cheese has a higher score than the control, it is still of lower quality than that of cows':

Average score

	Flavour and aroma	Body and texture	Total
Cows'	48	36	84
Buffaloes' control	42	30	72
Buffaloes' experimental	48	33	81

It is concluded that modifying the casein/fat ratio in buffaloes' milk and ripening it to an acidity of 0·21 % results in the production of better quality cheese. The modification of the ratio seems to be the important factor in this improvement. The differences between the average total score of control and experimental buffaloes' milk cheese in the second and third series were very similar, viz. 8 points and 9 points.

Moreover, owing to the fact that the moisture content of cheese exerts the most pronounced effect on its ripening and quality, and as prolonged ripening of milk causes the contraction of the curd, i.e. expulsion of whey, this factor must be excluded.

Table 22. *Average composition of cheese at intervals*

(Third series, Exps. nos. 7–9)

	Moisture (%)			Fat (%)			Total N (%)			Soluble N (%)			Non-protein N (%)			Salt (%)			Acidity (%		
Intervals	*C*	*B*	B_1	*C*	*B*	B_1	*C*	*B*	B_1	*C*	*B*	B_1	*C*	*B*	B_1	*C*	*B*	B_1	*C*	*B*	*B*
1 day	44·96	38·11	41·00	26·90	34·37	29·34	3·41	3·33	3·64	0·120	0·114	0·136	0·064	0·069	0·076	1·40	1·40	1·34	1·36	1·35	1·
1 week	44·10	37·42	39·99	—	—	—	3·46	3·37	3·70	0·208	0·178	0·238	0·126	0·122	0·162	—	—	—	1·62	1·62	1·
2 weeks	43·07	36·99	39·45	—	—	—	3·51	3·39	3·73	0·320	0·257	0·314	0·196	0·163	0·215	—	—	—	1·75	1·72	1·
4 weeks	41·89	35·89	38·42	—	—	—	3·60	3·45	3·81	0·519	0·410	0·511	0·349	0·263	0·328	—	—	—	2·05	1·90	2·
6 weeks	40·47	34·77	37·60	—	—	—	3·70	3·50	3·86	0·649	0·479	0·619	0·436	0·333	0·428	—	—	—	2·15	2·04	2·
8 weeks	39·09	33·88	36·46	29·86	36·76	31·60	3·78	3·56	3·94	0·741	0·542	0·719	0·560	0·410	0·543	1·57	1·53	1·48	2·02	2·04	2·

C = cows. *B* = control buffaloes. B_1 = modified ripened buffaloes.

Table 23. *Cheese-making data*

	Experiment 1			Experiment 2			Experiment 3		
	C	*B*	B_1	*C*	*B*	B_1	*C*	*B*	B_1
Acidity of milk (%)	0·185	0·170	0·170	0·205	0·180	0·180	0·185	0·185	0·185
Fat in milk (%)	3·70	4·70	4·70	3·00	3·95	3·95	3·50	4·45	4·45
Amount of starter (lb.)	¼	⅛	⅛	¼	⅛	⅛	¼	⅛	⅛
Casein/fat ratio	0·64	0·62	0·62	0·77	0·73	0·73	0·71	0·69	0·69
Time of ripening (min.)	45	180	180	30	105	105	30	30	30
Acidity at cutting (%)	0·130	0·110	0·110	0·135	0·105	0·105	0·125	0·105	0·105
Time of scalding (min.)	60	30	30	60	30	30	60	30	30
Temp. of scalding (° F.)	95	95	90	95	95	90	95	95	90
Time of pitching (min.)	5	60	80	10	95	95	85	120	120
Acidity whey drawn off (%)	0·145	0·150	0·145	0·150	0·150	0·145	0·145	0·150	0·150
Time of Cheddaring (min.)	185	175	185	120	155	160	165	180	180

C = cows.

B = modified buffaloes' scalded to 95° F. for 30 min.

B_1 = modified buffaloes' scalded to 90° F. for 30 min.

(*d*) *Effect of the temperature and rate of scalding on modified buffaloes' milk curd*

The results obtained in series (*c*) showed that modified buffaloes' milk cheese was still of lower moisture content than that of cows, and consequently cheese ripening, as judged by protein degradation and score, was also adversely affected. For these reasons, further attempts were made to manufacture buffaloes' curd of moisture content similar to that of cow's milk curd by modifying the period and temperature of scalding in three experiments.

Three vats were used, the first being cows' milk for comparison, while the other two vats were of buffaloes' milk, in which the casein/fat ratio was modified as in the previous series, and then renneted at an acidity of 0·19 %. The scalding period for these two vats was reduced from 60 to 30 min., while the temperature was raised to 95° F. in one vat and 90° F. in the other. Otherwise all vats followed the procedure usual for Derby cheese-making. Cheese-making details, which differed from one vat to another, are given in Table 23.

The analytical data in Table 23 show that when the period of scalding is reduced to 30 min. the moisture content of cheese is not noticeably affected. Comparing the moisture content of green cheese produced from the second vat in this experiment with the moisture content of green cheese produced from the modified buffaloes' milk in the second and third series of experiments, no great difference was observed, the average values being 39·35, 40·12 and 41·00 % respectively.

Low cooking temperature, on the other hand, effectively increased the moisture content of the cheese. The average value is 4·52 % higher for the third vat than for that of cheese produced from the second vat. Throughout the ripening period, the rate of water evaporation is slightly faster with the former, but the final average value of retained moisture is still higher in the former. The moisture content of cows' milk cheese, both green and ripened, is very close to that of the third vat, but differs considerably from that of buffalo cheese of the second vat (Table 24).

Table 24. *Average composition of cheese at intervals*

(Fourth series, Exps. nos. 10–12)

	Moisture (%)			Fat (%)			Total N (%)			Soluble N (%)			Non-protein N (%)			Salt (%)			Acidity (%)		
ervals	*C*	*B*	B_1	*C*	*B*	B_1	*C*	*B*	B_1	*C*	*B*	B_1	*C*	*B*	B_1	*C*	*B*	B_1	*C*	*B*	B_1
day	44·97	39·36	43·88	27·94	31·41	28·59	3·26	3·57	3·31	0·149	0·141	0·156	0·089	0·094	0·097	1·46	1·36	1·43	1·32	1·53	1·62
week	43·95	38·57	42·82	—	—	—	3·32	3·63	3·39	0·286	0·259	0·348	0·191	0·187	0·218	—	—	—	1·73	1·90	2·07
weeks	42·99	37·49	41·82	—	—	—	3·40	3·68	3·45	0·419	0·367	0·428	0·273	0·242	0·290	—	—	—	2·03	2·21	2·25
weeks	41·72	36·28	40·56	—	—	—	3·47	3·75	3·51	0·535	0·487	0·562	0·413	0·367	0·403	—	—	—	2·28	2·33	2·47
weeks	40·80	34·84	39·25	—	—	—	3·52	3·85	3·60	0·688	0·623	0·707	0·557	0·474	0·520	—	—	—	2·29	2·31	2·40
weeks	38·72	33·27	37·32	31·11	34·42	31·98	3·65	3·93	3·71	0·838	0·731	0·849	0·688	0·571	0·651	1·64	1·54	1·58	2·42	2·37	2·47

C = cows. *B* = modified buffaloes' scalded to 95° F. B_1 = modified buffaloes' scalded to 90° F.

This variation in the moisture content is accompanied by acid development and protein degradation values, which run at approximately the same level for cows' milk cheese and that produced from buffaloes' milk in the third vat. At the end of ripening, the average values are acidity 2·42 and 2·47 %, soluble N 0·838 and 0·849 %, non-protein N 0·688 and 0·651 % respectively.

The effect of these treatments on cheese quality is shown below. Cows' milk cheese obtained a markedly higher score than the buffalo cheese of the second vat, but differed little from buffalo cheese of the third vat. The difference in the total score is 12 points in the first case, but only 3 points in the second:

Average score

	Flavour and aroma	Body and texture	Total
Cows'	54	35	89
Buffaloes' scalded to 95° F.	47	30	77
Buffaloes' scalded to 90° F.	52	34	86

Three confirmatory trials were made, using cows' milk and buffaloes' milk as in the first and third vats described above. The following table gives the average composition and score of the two kinds of cheese at the end of ripening:

Average composition and score of mature cheese

(Three confirmatory experiments)

	Moisture (%)	Fat (%)	Total N (%)	Soluble N (%)	Non-protein N (%)	Salt (%)	Acidity (%)	Score points: Flavour and aroma	Score points: Body and texture	Score points: Total
Cows' milk cheese	38·30	30·67	3·75	0·854	0·657	1·78	2·85	53	36	89
Buffaloes' milk cheese	37·46	31·33	3·81	0·870	0·660	1·77	2·86	51	36	87

GENERAL CONCLUSIONS

Modification of the casein/fat ratio in buffaloes' milk had a beneficial effect upon the quality of the resultant cheese. It increased the capacity of the curd to retain moisture, and permitted more rapid and extensive protein degradation than in cheese produced from

unmodified milk of the same batch. Prolonged ripening of this milk, besides introducing the possibility of taint development, did not improve moisture retention in the curd. The development of acidity during cheese ripening was probably more dependent upon milling acidity than upon renneting acidity.

By modifying the casein/fat ratio of milk and scalding at a lower temperature (90° F.) and for a shorter interval than usual (30 min.), the moisture content of the resultant curd and also that of the mature cheese increased to approximately the level of cows' milk cheese, the chemical properties and quality also approximated to those of cows' milk cheese.

As further evidence for the improvement in the composition and quality of buffalo cheese, samples of mature cheese from the confirmatory experiments were graded by various colleagues unaware of their identity and origin. Of sixteen persons, eleven gave a higher score for the modified buffaloes' milk cheese of the second vat than for that of cows', while four out of the remaining five gave a lower score for the former and only one put them in the same grade.

It is, however, obvious that, in spite of these successful results, further studies are needed. Work of this kind on hard cheese has only recently begun in Egypt, and this preliminary study may assist further investigations.

SUMMARY

1. Hard cheese of Cheddar, Derby and Manfis types was made from cows' milk and from buffaloes' milk. Buffaloes' milk curd of the Derby type was also milled at different acidities.

2. Initial acidity was higher and rate of acid development was greater in cows' milk and curd, whilst loss of fat in the whey was lower.

3. Cows' milk cheese contained more moisture, more total, soluble, and non-protein nitrogen, but less fat than buffaloes' milk cheese.

4. During ripening, the rate of acid development and protein degradation was greater in cows' milk cheese, whilst loss of moisture was usually smaller.

5. Buffaloes' milk cheese was inferior in grading quality to cows' milk cheese.

6. Attempts to improve the quality of buffaloes' milk cheese were made by altering the Derby cheese-making process as follows:

(*a*) ripening milk longer before renneting;

(*b*) modification of the milk casein/fat ratio;

(*c*) combining (*a*) and (*b*);

(*d*) modification of the casein/fat ratio plus scalding of the curd at a lower temperature and for a shorter time.

7. The last treatment (*d*) proved most effective and yielded cheese of very similar chemical composition and grading quality to that obtained from cows' milk.

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479. STUDIES ON THE BIOLOGICAL VALUE OF THE PROTEINS (NITROGEN) OF DRIED SKIM MILK: EFFECTS OF THE ADDITION OF CERTAIN AMINO-ACIDS, OF AGE OF RAT AND OF LEVEL OF PROTEIN INTAKE

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Henry & Kon (1) and Henry, Kon & Rowland (2) found that the biological value of the proteins (B.V.) of dried skim milk deteriorates on storage. Extensive experiments (3) to elucidate the cause led to a number of observations the significance of which has now been further investigated.

(1) The deterioration proved largely due to inactivation of lysine by combination of its ϵ-amino group in a Maillard reaction (3) and the B.V. could be restored to its original value by addition of lysine. The addition of lysine to control, unchanged, milk caused a lowering of the B.V. A plausible explanation was that the added lysine was in excess of the requirements of the animals and was, therefore, rejected (3). To check this explanation D-lysine was now used, as it is generally agreed that this unnatural isomer is not utilized by the rat. Thus Berg (4) showed that, for growth, it did not replace L-lysine; Foster, Rittenberg & Schoenheimer (5) and Ratner, Weissman & Schoenheimer (6) proved its uselessness by studies with the amino-acid labelled with deuterium alone or in combination with ^{15}N, and Neuberger & Sanger (7) as a result of measurements of urinary excretion.

(2) Our earlier work (3) showed that the proteins of dried skim milk were slightly, but significantly, more injured on storage in air-pack than in gas-pack. An experiment by the rat-growth method of Osborne, Mendel & Ferry (8) indicated that this may have been due to loss or inactivation of histidine (3). This experiment was now repeated by the more precise balance-sheet method (9, 10).

(3) The adult rat requires less of most essential amino-acids than the young growing rat. In particular, its need for lysine is very markedly reduced (11, 12) and there is some doubt whether histidine is required at all by the adult (13, 14). Previous experiments in this laboratory (2) with bread, known to be deficient in lysine, gave a significantly higher B.V. for adult than for young animals. However, with two different samples of milk there was no difference between young and adult rats. It is possible that, as pointed out by Bricker & Mitchell (15), the milk proteins given in these experiments at an 8 % level supplied essential amino-acids in excess of the adult rat's needs. For this reason these experiments were repeated with milk protein forming only 4 % of the diet. Some other experiments with milk in which lysine and histidine were the limiting amino-acids for the young rat were also repeated with adults.

EXPERIMENTAL

General procedure

Animals and technique. In all experiments described below the B.V. and true digestibility of the proteins (T.D.) of dried skim milk samples were determined by the balance-sheet method of Mitchell (9, 10); each test was carried out on twelve female rats in litter-mate

comparisons. In Exps. 1 and 2 the protein efficiency ratio (P.E.) of the milks was determined by the rat-growth method (8) on groups of six litter-mate male rats. Details of the management and feeding of the rats will be found in our earlier publications (3, 16).

The basal diet had the following percentage composition: rice starch 64, sugar 12, potato starch 10, margarine fat 10, salts (17) 4. In the experimental diets the milk samples were added to the basal diet in place of an equal weight of rice starch. Details of the diets and their nitrogen content are given in Table 1. In all experiments the milks supplied 8 % protein (N × 6·38); in Exp. 4 they were tested also at a 4 % protein level.

Table 1. *Quantity and source of protein and percentage nitrogen in the experimental diets*

Diet no.	Description of spray-dried skim milk tested*	Milk in diet (%)	N in diet (%)
	Exp. 1		
293	M	23·4	1·258
301	M + 1·25 % L-lysine	22·5	1·257
302	M + 1·25 % D-lysine	22·5	1·256
	Exp. 2		
308	H	21·8	1·277
308 A	H, gas-packed, stored at 37° C. for 60 days	21·8	1·249
308 B	H, air-packed, stored at 37° C. for 60 days	21·8	1·238
309	H, air-packed, stored at 37° C. for 60 days, + 0·5 % L-histidine	21·7	1·252
	Exp. 3		
308	H	21·8	1·287
326	H + 0·5 % L-histidine	21·7	1·257
327	H, gas-packed, stored at 37° C. for 60 days, + 1·25 % L-lysine	22·3	1·283
328	H, gas-packed, stored at 37° C. for 60 days, + 1·25 % L-lysine + 0·5 % L-histidine	22·1	1·281
	Exp. 4		
323	H	10·6	0·642
323 A	H, air-packed, stored at 37° C. for 60 days	10·6	0·633
308	H	21·8	1·284
308 B	H, air-packed, stored at 37° C. for 60 days	21·8	1·234

* M = milk with 4·7 % moisture, H = milk with 7·5 % moisture.

Milk powders used. The M, medium-moisture, control milk prepared for the earlier tests (3) was used in Exp. 1. Between manufacture and use it was stored at −20° C. A fresh batch of high-moisture (H) milk containing about 7·5 % water was prepared, as before, for use in Exps. 2–4. Part of this milk was stored at −20° C. until needed as control sample. The remainder of the powder was stored in air or nitrogen for 60 days at 37° C. and then at −20° C. until needed for the tests. To accentuate the difference previously found between air- and gas-pack, the air-pack sample was packed in 'extra air' (cf. (3)).

Statistical procedure. In examining the statistical significance of treatment differences for the B.V. and T.D. of the milk samples, an analysis of variance was carried out after angular transformation (18, 19) of the observed values. The error mean squares of separate litters were tested for homogeneity (20), and where the χ^2 values were significant ($P < 0{\cdot}05$) weighted means have been given. Mean values, derived from the means of transformed data, together with their 5 % fiducial limits, are quoted in Table 2. For consistency with previously published results, arithmetic means of the original, untransformed values are also presented. In Exp. 4, where the tests were done with rats when young and again at maturity, one animal died in the interval between the two tests; the equations given by

Yates (21) were first solved to estimate the diet means in the depleted litter and the methods quoted in that paper were then applied.

Exp. 1. *The effect of the addition of* 1·25 % D- *or* L-*lysine to control milk*

The B.V., T.D. and P.E. of the M control milk (3) alone or supplemented with 1·25 % L- or D-lysine were determined on young rats at an 8 % level of protein intake. Lysine was added as the monohydrochloride dihydrate; the D-isomer was prepared by the Walden inversion method described by Neuberger & Sanger (22).

Exp. 2. *Comparison of stored air-pack and gas-pack samples of milk; effect of addition of* 0·5 % L-*histidine to the air-pack sample; effect of age of rat*

The B.V., T.D. and P.E. of the following samples of milk were determined on young rats at an 8 % level of protein intake: H, control; H, gas-pack, stored at 37° C. for 60 days; H, air-pack, stored at 37° C. for 60 days; H, air-pack, stored at 37° C. for 60 days and then supplemented with 0·62 % L-histidine monohydrochloride (0·5 % histidine). At the end of this test the rats, except those used for the P.E. tests, were given our stock-colony diet (23), with 10 % of the whole wheat replaced by wheat germ, until they were 4½ months old. The B.V. and T.D. of the milks were then determined again.

Exp. 3. *Effect of the addition of* 0·5 % L-*histidine to control milk or to stored gas-pack milk supplemented with* 1·25 % L-*lysine*

The B.V. and T.D. of samples of the H control milk and of the H gas-pack milk stored for 60 days at 37° C. and supplemented with 1·25 % L-lysine were determined on young rats at an 8 % level of protein intake. Both samples were tested alone and supplemented with 0·5 % L-histidine.

Exp. 4. *Effect of level of protein intake from control milk and from stored air-pack milk; effect of age of rat*

The B.V. and T.D. of the H control milk and the H milk stored in air-pack for 60 days at 37° C. were determined on young rats at 4 and 8 % levels of protein intake. At the end of this test, as in Exp. 2, the animals were given the stock-colony diet and the experiment was repeated when they were 6 months old.

RESULTS

Figures for the B.V. and T.D. obtained in the four experiments are given in Table 2 and values for the P.E. obtained in Exps. 1 and 3 are shown in Table 3. The results of the statistical analysis are given in Tables 4, 5, 6 and 7.

Exp. 1. *The effect of the addition of* 1·25 % D- *or* L-*lysine to control milk*

The addition of D- or L-lysine to the M control milk lowered its B.V. from 89·2 to 84·7 and 85·9 respectively, in good agreement with our previous work (3), where the addition of 1·25 % L-lysine to milk from the same bulk (see p. 217) lowered the B.V. from 87·6 to 83·7. D-Lysine is not utilized by the rat (4, 5, 6, 7), and as its natural isomer depressed the B.V. to the same extent it may be concluded, as previously suggested, that the added L-lysine was not required by the rat. A recalculation of the B.V. on the assumption that this added lysine was excreted in the urine gives values of 88·1 and 90·1 for milk supplemented with

Table 2. *Mean results for groups of twelve rats for the biological values and true digestibilities of the proteins* (N × 6·38) *of the milks studied in the various experiments*

Exp. no.	Age of rats at start (days)	Weight of rats at start (g.)	Diet no.	Biological value: Mean of observed results	Biological value: Value derived from transformed results*: Mean	Biological value: Value derived from transformed results*: 5 % fiducial limits	True digestibility: Mean of observed results	True digestibility: Value derived from transformed results*: Mean	True digestibility: Value derived from transformed results*: 5 % fiducial limits
1	24	50–61	300	89·2	90·0	(88·4; 91·4)	93·1	93·5†	(93·1; 93·8)
			301	85·9	86·3	(84·5; 88·0)	92·6	92·5†	(92·2; 92·9)
			302	84·7	85·2	(83·4; 86·9)	92·9	95·2†	(94·9; 95·4)
2	24	56–62	308	88·2	88·6	(87·0; 90·0)	92·9	92·7†	(91·5; 94·1)
			308A	66·2	66·3	(64·0; 68·3)	85·1	84·7†	(82·9; 86·6)
			308B	63·1	63·2	(60·7; 65·3)	84·1	83·6†	(81·6; 85·5)
			309	66·4	66·5	(64·2; 68·7)	87·3	86·9†	(85·1; 88·6)
	135	198–257	308	64·2	63·7†	(62·2; 65·0)	92·9	93·5	(92·3; 94·7)
			308A	68·2	67·7†	(66·3; 69·0)	89·9	90·3	(88·8; 91·6)
			308B	64·8	64·7†	(63·2; 66·0)	87·9	88·3	(86·6; 89·8)
			309	65·8	64·2†	(62·8; 65·7)	88·4	88·6	(86·9; 90·0)
3	25	56–61	308	79·6	80·1	(78·9; 81·4)	91·5	91·8†	(90·7; 93·0)
			326	80·6	80·9	(79·6; 82·4)	90·9	90·7†	(89·4; 91·9)
			327	75·7	76·0	(74·6; 77·4)	88·8	88·4†	(87·0; 89·8)
			328	77·5	77·7	(76·3; 79·1)	88·5	88·7†	(87·2; 90·0)
4	25	58–66	323	82·6	83·6	(80·4; 86·6)	100·0	100·0	(99·8; ‡)
			323A	71·2	71·6	(67·7; 75·3)	98·9	99·7	(99·1; 100·0)
			308	85·0	85·2	(82·1; 88·1)	99·5	99·9	(99·5; ‡)
			308B	58·9	59·0	(54·8; 63·2)	91·9	92·7	(90·6; 94·7)
	186	247–311	323	91·2§	93·9§	(90·9; 96·4)	97·9§	99·8§	(99·2; 100·0)
			323A	92·1§	94·2§	(91·3; 96·6)	97·4§	98·2§	(96·8; 99·1)
			308	74·6§	73·5§	(68·2; 78·4)	92·4§	93·6§	(91·4; 95·5)
			308B	70·3§	70·2§	(64·8; 75·3)	91·2§	93·2§	(91·0; 95·2)

* See p. 217, Tables 4, 5, 6, 7.
† Weighted mean, see p. 217.
‡ The upper 5 % fiducial limit is outside the range of the transformation.
§ Mean for 11 rats, see p. 217.

Table 3. *Mean results for groups of six rats for the protein* (N × 6·38) *efficiency ratios of the milks studied in Exps.* 1 *and* 3

Exp. no.	Age of rats at start (days)	Weight of rats at start (g.)	Diet no.	Diet intake (g.)	Gain in weight (g.)	Protein efficiency ratio: Value	Protein efficiency ratio: Pooled S.E.
1	25–26	57–65	300	297·00	70·3	2·96	±0·084
			301	279·85	67·2	2·98	
			302	277·12	64·7	2·91	
2	24–27	56–77	308	288·59	71·5	2·87	±0·095
			308A	238·44	35·5	1·84	
			308B	256·09	33·3	1·62	
			309	257·15	37·2	1·73	

Table 4. *Exp.* 1. *Transformed mean values for groups of twelve rats for the biological values and true digestibilities of the proteins of the milks studied, and statistical significance of the differences*

Diet no.	Transformed mean: Biological value	Transformed mean: True digestibility*	Significance of differences between means: Dietary difference tested	Significance of differences between means: P: Biological value	Significance of differences between means: P: True digestibility
300	71·6	75·2	300 – 301	<0·01	<0·01
301	68·3	74·1	300 – 302	<0·01	<0·001
302	67·4	77·3	301 – 302	>0·05†	<0·001
S.E. (8 D.F.)	±0·62	±0·16			

* Weighted means, see p. 217. † Not significant.

the D- or L-form. These figures are not significantly different from that of 89·2 obtained for the unsupplemented milk.

In agreement with our earlier findings (3) the addition of 1·25 % lysine did not affect the P.E. of the milk, probably because the method is not sensitive enough to record the effect of this quantity. The T.D. was also unchanged.

Exp. 2. *Comparison of stored air-pack and gas-pack samples of milk; effect of addition of* 0·5 % *L-histidine to the air-pack sample; effect of age of rat*

The results with young rats again showed a small difference in B.V. of the air- and gas-pack samples of milk that just failed to reach conventional statistical significance (cf. (3)). The difference was eliminated by the addition of 0·5 % L-histidine to the air-pack sample. As in our earlier experiments (1) the T.D. of the milk was significantly lowered by storage in both packs. The addition of L-histidine caused a small but significant increase in the T.D. of the stored air-pack milk. The P.E. values for the milks followed the same pattern as the B.V.; all were significantly lower than the value obtained for the control milk, but the differences between the three stored samples were too small for statistical significance.

Table 5. *Exp.* 2. *Transformed mean values for groups of twelve rats when young and when adult for the biological values and true digestibilities of the proteins of the milks studied, and statistical significance of differences between diets and between young and adult animals*

	Biological value				True digestibility			
	Value		Difference : Adult-young		Value		Difference : Adult-young	
Diet no.	Young rats	Adult rats*	S.E. (2 D.F.)	*P*	Young rats	Adult rats	S.E. (2 D.F.)	*P*
308	70·2	52·9	±0·99	<0·001	74·4	75·3	±1·21	>0·05†
308A	54·5	55·4	±0·97	<0·01	67·0	71·8	±1·10	<0·01
308B	52·6	53·5	±1·15	<0·01	66·1	70·0	±2·15	>0·05†
309	54·6	53·2	±2·63	<0·001	68·8	70·2	±0·80	>0·05†
S.E. (18 D.F.)	±0·65	±0·40			±0·70	±0·67		

Significance of differences between means

Dietary difference tested	*P* (Biological value)		*P* (True digestibility)	
	Young rats	Adult rats	Young rats	Adult rats
308 – 308A	<0·001	<0·001	<0·001	<0·01
308 – 308B	<0·001	>0·05†	<0·001	<0·001
308 – 309	<0·001	>0·05†	<0·001	<0·001
308A – 308B	>0·05†	<0·01	>0·05†	>0·05†
308A – 309	>0·05†	<0·01	>0·05†	>0·05†
308B – 309	<0·05	>0·05†	<0·05	>0·05†

* Weighted mean, see p. 217. † Not significant.

The B.V. of the control milk was very much lower for the adult rats (64·2) than when they were young (88·2); it declined in fact to the values obtained with the young rats for the stored, deteriorated powders. For these, however, the B.V. remained the same irrespective of the age of the rat. With the T.D. of the milks the effect of the age of the rat was less striking. The value for the control milk, 92·9, was the same for young and adult animals. For the stored samples higher values, though still inferior to that for the control milk, were obtained with old than with young rats, but the differences were significant only for the unsupplemented stored milks.

Exp. 3. *Effect of the addition of* 0·5 % L-*histidine to control milk or to stored gas-pack milk supplemented with* 1·25 % L-*lysine*

The addition of 0·5 % L-histidine had no effect on the B.V. or T.D. of the control milk and of the lysine-supplemented milk stored in gas-pack. Histidine was, therefore, not a limiting amino-acid for these milks. The quantity added was too small to depress the B.V. like the addition of lysine. In contrast to our earlier findings the B.V. of the stored gas-pack milk supplemented with 1·25 % L-lysine was significantly lower than that of the control milk. As already mentioned, a fresh batch of milk was prepared for the present tests and it seems probable that different amounts of lysine may have become inactivated

Table 6. *Exp.* 3. *Transformed mean values for groups of twelve rats for the biological values and true digestibilities of the proteins of the milks studied, and statistical significance of the differences*

	Transformed mean		Significance of differences between means		
				P	
Diet no.	Biological value	True digestibility*	Dietary difference tested	Biological value	True digestibility
308	63·5	73·4	308 – 326	>0·05†	>0·05†
326	64·1	72·2	308 – 327	<0·001	<0·001
327	60·7	70·1	308 – 328	<0·05	<0·01
328	61·8	70·3	326 – 327	<0·001	<0·05
			326 – 328	<0·01	<0·05
S.E. (18 D.F.)	±0·46	±0·59	327 – 328	>0·05†	>0·05†

* Weighted means, see p. 217. † Not significant.

in the two batches of milk. On two occasions in our earlier work (1) the B.V. of the gas-pack milk stored for 60 days at 37° C. was some 80 % of that of the control milk and the addition of 1·25 % L-lysine increased it to within 1 % of that value. The loss of some 25 % (from 88·2 to 66·2) in B.V. on storage in Exp. 2 indicates a greater inactivation of lysine and 1·25 % of this acid was probably insufficient to restore the B.V. to normal.

Exp. 4. *Effect of level of protein intake from control milk and from stored air-pack milk; effect of age of rat*

For young animals the B.V. of the control milk was markedly higher than that of the stored milk at both the 4 and the 8 % level of intake. The value for the control milk itself was not significantly different at the two levels of intake; that for the stored milk, however, was significantly higher at the 4 than at the 8 % level. The values obtained with the adult rats were markedly lower at the 8 than at the 4 % level, for which very high values of 91 and 92 were obtained; but at neither was the difference between the control and stored milk samples significant. The age differences were less marked in this experiment for the 8 % protein diet with control milk than they were in Exp. 2. For the stored milk at this level a significantly higher value was obtained with adult than with young rats, whereas in Exp. 2 the value for this sample was independent of the age of the rat.

Somewhat lower values for the T.D. of all milk samples tested were obtained with adult than with young rats, but the difference was significant only for the control milk at the 8 % level. With young rats there was no difference in the T.D. of the two milk samples at the 4 % level or for the control milk at 8 %, but a significantly lower value was obtained

for the stored sample at the higher level. With the adult animals, no difference attributable to storage was detected at either level but, irrespective of storage, the digestibility was significantly better at the lower level.

Table 7. *Exp.* 4. *Transformed mean values for groups of twelve rats when young and when adult for the biological values and true digestibilities of the proteins of the milks studied, and statistical significance of differences between diets and between young and adult animals*

	Biological value				True digestibility			
	Value		Difference : Adult-young		Value		Difference : Adult-young	
Diet no.	Young rats	Adult rats	S.E. (2 D.F.)	*P*	Young rats	Adult rats	S.E. (D.F.)	*P*
323	66·1	75·7	±2·13	<0·05	90·0	87·3	±1·23	>0·05‡
323A	57·8	76·1	±3·58	<0·05	86·8	82·2	±2·28	>0·05‡
308	67·4	59·0	±1·38	<0·05	88·1	75·4	±1·83	<0·05
308B	50·2	56·9	±0·68	<0·02	74·4	74·9	±2·19	>0·05‡
S.E.	±1·12*	±1·56†			±1·08*	±1·12†		

Significance of differences between means

Dietary difference tested	*P*		*P*	
	Young rats	Adult rats	Young rats	Adult rats
323 – 323A	<0·001	>0·05‡	>0·05‡	<0·01
323 – 308	>0·05‡	<0·001	>0·05‡	<0·001
323 – 308B	<0·001	<0·001	<0·001	<0·001
323A – 308	<0·001	<0·001	>0·05‡	<0·01
323A – 308B	<0·001	<0·001	<0·001	<0·001
308 – 308B	<0·001	>0·05‡	<0·001	>0·05‡

* 18 D.F. † 15 D.F., see p. 217. ‡ Not significant.

DISCUSSION

We have now obtained experimental support for two suggestions put forward in our earlier work (3).

First, proof is available that lysine added to a control, undeteriorated sample of milk is not utilized by the rat and that its excretion in the urine causes an apparent lowering of the B.V. of the milk. Secondly, the small difference in B.V. between samples of milk stored in air or in inert gas is due to loss or inactivation of histidine in the air-pack sample, probably as a result of oxidative changes. The addition of histidine did not improve the B.V. of undeteriorated milk or of milk that had been stored in the absence of oxygen; histidine is therefore not a limiting factor in such milks.

The amino-acid requirements of the adult rat for maintenance are, in general, less than those of the young rat which needs them also for growth. The sulphur amino-acids are a notable exception (12,24), as they are needed by the young and mature rat alike for the growth of fur (24,25).

It has been established that arginine, essential for the young, is not needed in the diet of the adult rat (13,26,27). Wolf & Corley (26) and Wissler, Steffee, Frazier, Woolridge & Benditt (27) consider that the remaining nine amino-acids essential for the young rat are also required by the adult, but Burroughs, Burroughs & Mitchell (13) are of the opinion that in addition to arginine the adult rat can dispense also with lysine, leucine, histidine and phenylalanine.

More recently Mitchell (12) has modified his view about lysine and, in agreement with

Neuberger & Webster (11), suggests that it may be required but in quantities relatively much less than of other amino-acids essential for the adult rat. He thus supports the estimate of Neuberger & Webster (11) that the adult rat requires, for maintenance of weight, some 16 mg. lysine daily, compared with 100 mg. needed by the young animal for growth. In our experience rats when mature did not detect the loss of lysine in the stored samples of milk at whatever level they were given. Hence their need for this amino-acid must have been relatively very much less than when they were young.

Table 8. *Biological values of proteins from different sources determined on growing and adult rats at different protein levels*

Protein source	Protein in diet (%)	Biological value: Young rats	Biological value: Adult rats	Reference
Limiting amino-acids: cystine and methionine				
Dried skim milk	4	83	91	This paper, Exp. 4
	8	85	75	
	8	88	64	This paper, Exp. 2
	5	—	78	(28)
	8	84	64	
	2–4	—	89	(15)
	4–5	—	96	(12)
	10	84	—	
Casein	2–4	—	51	(24)
	10	69	—	
Limiting amino-acids: cystine, methionine and lysine				
Dried skim milk, after 5 years' storage	8	71	73	(2)
Dried skim milk, poor commercial sample*	8	55	57	
High moisture dried skim milk stored 60 days in gas-pack	8	66	68	This paper, Exp. 2
High moisture dried skim milk stored 60 days in air-pack, +0·5 % histidine	8	66	66	
Limiting amino-acids: cystine, methionine, lysine and histidine				
High moisture dried skim milk stored 60 days in air-pack	4	71	92	This paper, Exp. 4
	8	59	70	
	8	63	65	This paper, Exp. 2
Limiting amino-acid: lysine				
Bread made from 85 % extraction flour	8	49	56	(2)
	8	55	65	
Wheat gluten	2–4	—	65	(24)
	10	40	—	

* Probably also deficient in other amino-acids.

Table 8 lists B.V. obtained by us and by other workers with young and adult rats. Broadly speaking they support the view of Mitchell & Beadles (24) that differences in B.V. obtained with young and mature rats are roughly correlated with the amino-acid content of the proteins and with the specific requirements of the different ages.

For normal, undeteriorated, samples of dried skim milk, B.V. obtained in different laboratories at the same level of intake agreed well in tests with young or with mature animals (Table 8). With the milk proteins of high B.V. adult rats may waste some nitrogen at the 8 % level, hence the values for them are as a rule higher for lower proportions of these proteins in the diet.

Mitchell & Beadles (24) consider that the very low B.V. of 51 they obtained for casein on adult rats at a 2–4 % level expressed the increased need of such animals for cystine and methionine. With milk we found no such lowering at low levels of intake, in fact at the

4 % level we obtained higher values with adult rats than those for young rats on either the 4 or the 8 % level (Exp. 4). Milk and casein are equally deficient in methionine, but the cystine deficiency is more pronounced in casein (29). Our results indicate that in milk the sulphur amino-acids are not a limiting factor for the adult rat. The lower value for adult rats than for young found by us and by Sumner (28) at the 8 % level is probably connected with the lowered lysine requirement of the adult, leading to excess of this amino-acid in the diet. It is possible that other acids may also be in excess. Our results show that with 4 % of milk protein in the diet the amino-acid balance may be more favourable for the adult than for the young rat both with deteriorated and intact milk. With stored milk samples, in which lysine and, to a much smaller extent, histidine, were the limiting amino-acids for the young rat, the values obtained at the 8 % level were independent of the age of the rat in Exp. 2, but higher with adult than with young animals in Exp. 4. With bread, markedly deficient in lysine, we obtained higher values with adult than with young rats (2). Similarly, Mitchell & Beadles (24) found a very much higher value for wheat-gluten with adult than with young rats (Table 8). These authors, however, gave 10 % protein to young rats but only 2–4 % to the adult animals.

It would appear that our findings with young and adult rats reflect the known differences in their requirements for essential amino-acids.

SUMMARY

1. It has been shown that the biological value of undeteriorated dried skim milk is depressed to the same extent by the addition of 1·25 % L-lysine as by the addition of 1·25 % D-lysine. The latter is not used by the rat. It is therefore concluded that the added L-lysine is surplus to the animal's needs and that the apparent lowering of the biological value is due to excretion of the lysine in the urine.

2. The slightly greater loss in the biological value of milk stored in air-pack compared with gas-pack was eliminated by the addition of 0·5 % L-histidine to the former. Histidine is not a limiting amino-acid in the control or stored gas-pack milks for either young or adult rats.

3. A lower biological value was found for the control milk with adult than with young rats at 4 and 8 % levels of intake. No further lowering in the biological value of the stored milk, deficient in lysine, was observed with adult rats, the value obtained for this milk being independent of the age of the rat. These results are in keeping with the known lowered requirements of the adult rat for lysine.

4. The significance of these findings in relation to the known amino-acid requirements of young and adult rats is discussed.

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REVIEWS OF THE PROGRESS OF DAIRY SCIENCE

SECTION C. DAIRY CHEMISTRY AND PHYSICS

PART I. CHEMISTRY AND PHYSICAL CHEMISTRY
I. Composition of milk and milk products:
(*a*) Milk
(*b*) Butter
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II. Chemistry of milk constituents:
(*a*) Fat
(*b*) Nitrogenous constituents and sugars
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III. Physical chemistry
IV. Defects of milk and milk products:
(*a*) Adulteration and its detection
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V. Analysis of milk and milk products:
(*a*) Milk
(*b*) Butter and butterfat
(*c*) Cheese
(*d*) Dried milk, etc.
References:
(*a*) Arranged numerically
(*b*) Arranged alphabetically
PART II. PHYSICS (RHEOLOGY)

PART I. CHEMISTRY AND PHYSICAL CHEMISTRY

This review deals with the progress of dairy chemistry from 1947 to about the middle of 1950. It includes those papers of 1947 that were not covered by the previous Review(1).

I. COMPOSITION OF MILK AND MILK PRODUCTS

(*a*) *Milk*

Cow's milk

The increased interest that has been taken of recent years in the compositional quality of milk supplies has been maintained and the subject has been dealt with in several countries. Provan(2) has investigated the composition of the milk delivered to creameries in various parts of England and Wales, and has contributed data on the trends in composition that have occurred over a period of years and on the seasonal and breed variations. Laboratory records for the years 1923–46 which were available for a group of creameries in four midland counties showed that since 1930 the average solids-not-fat content had fallen from about 8·90 to 8·65 %, the latter level being similar to that now found in many areas. Data from the Milk Marketing Board Creameries for the years 1945–7 showed that the fat content of milk follows a simple seasonal trend, being highest in November and lowest in May, but the seasonal changes in solids-not-fat are more complex, and vary for different parts of the country. The possible causes of these seasonal and regional differences are discussed, and also the incidence of producers supplying genuine milk below the presumptive legal standard of 8·5 % solids-not-fat. The trend in the compositional quality of milk since the earlier years of the century has also been discussed by Kay(3, 4) and Rowland(5), who have indicated the genetic, nutritional and other factors involved. Bartlett & Kay(6) have reviewed the legislation concerning the significance of the trend in, and the factors influencing, the composition of milk.

Grigg(7), in a similar manner, has studied the variations in the composition of the milk supply to the city of Christchurch, New Zealand, over the period 1926–45. The fat content, apart from a temporary rise in 1935–7, has not changed, but the solids-not-fat content has declined from 8·95–9·00 to 8·70–8·75 %, and this is attributed partly to an increase in the proportion of cows which, by their breeding, are potentially yielders of lower quality milk, and partly to an increasing tendency to a subnormal plane of nutrition at certain times. Nefdt & McNaughtan(8) have surveyed the solids-not-fat content of Kenya milk, collecting morning and evening samples once

from each of sixty-seven herds covering various breeds. Of these samples 21 % contained less than 8·5 % solids-not-fat, and 7 % less than 8·25 %; Friesian samples were the poorest, with 59 and 25 % respectively below these values.

Bakalor(9) has continued his investigations on the composition of South African milk, dealing with the influence of monthly variations in air temperature and rainfall, and with the relationships between the percentages of fat, solids-not-fat, protein, lactose and ash. No correlation was found between the monthly levels of fat or solids-not-fat and the mean air temperature of the same months, but there was a positive correlation between air temperature and the fat content of the milk produced 3–4 months later—an interesting observation that is, as yet, unexplained. Rainfall had no apparent influence on fat content but was, as might be expected, directly related to the solids-not-fat content. Among the data on the relation between the milk constituents it is surprising to find that even of the herd samples of milk testing as high as 4·0–4·5 % of fat, about 50 % were likely to contain less than the standard of 8·5 % solids-not-fat. In a later paper Bakalor(10) discusses the composition of the Cape Town City milk supply and the possible causes of the decline in fat and solids-not-fat content observed over the last ten years. Janse(11) has compared the fat and solids-not-fat contents of milk in the Friesland province of the Netherlands in 1927–8 with those of 1948–9, and Koestler(12) has reviewed the composition of milk in Switzerland at different times of the year and its bearing on cheese yields. In Australia, Cullity, Hood & Needham(13) have made a preliminary investigation of the seasonal variations in the composition of the milk of a herd with a reputation for substandard fat and solids-not-fat.

The variations in the constituents of milk under Arizona conditions have been studied by Davis, Harland, Caster & Kellner(14), who report the variation with breed, season and stage of lactation in the detailed composition of the milk of individual cows (eleven Jersey, eleven Guernsey and twelve Holstein) and of herds (two Jersey, one Guernsey, three Holstein and five of mixed breeds). Honer & Herzer(15) have made a study of the composition of milk produced in different areas of Mississippi. El-Sokkary & Hassan have investigated the fat, solids-not-fat, total protein and casein contents(16), and the lactose and chloride contents(17) of the milk of Egyptian cows and buffaloes; and a group of Indian workers(18) have made a comprehensive study of the detailed composition and freezing-point of the milk of Indian cows and buffaloes.

The amount and fat content of the residual milk left in the cow's udder after normal milking have been investigated by Dodd & Foot(19), who, immediately after the milking, injected cows with oxytocin and continued milking to obtain the so-called super-strippings. The average results for eight cows so treated on one morning per week for 16 weeks were that the residual milk contained 13·4 % of fat, and represented 22 % of the total milk and 54 % of the fat present in the udder. King(20) has discussed the various factors which affect the fat content of milk, stressing the significance of udder pressure, and Morrison, Erb & Shaw(21) have discussed the effect of oestrus and gestation. Van Rensburg(22) has investigated the secretion of milk of abnormal composition, akin to that of mastitis milk, by quarters believed to be free of known pathogens. Khambatta & Dastur(23) have followed the changes in the composition of raw and boiled cow's and buffalo's milk during souring.

The relation of the type and quantity of roughage and grazing to the fat content of milk has been dealt with in a valuable paper by McClymont(24), who has reviewed the small amount of literature on the subject and given his field observations in Australia on the effect of lush grazing plus concentrates, low roughage rations, and oat grazing in depressing the fat percentage. He also describes controlled experiments demonstrating a considerable depression of the percentage by grazing on short lush pasture, or young oats, supplemented with concentrates.

Tangl & Szarka(25) report that the feeding of 5 g. of fresh yeast per cow daily increased the fat content of the milk of some cows by up to 0·8 %, although others did not respond. Dried yeast did not have this effect. An increase in the fat content of the milk of some cows was also obtained when the diet was supplemented with 20 mg. of vitamin B_1 and 6 mg. of vitamin B_2. Lutz(26) has conducted an experiment on the effect of inadequate feeding on milk yield and composition; reducing the average starch equivalent and digestible protein from 6·8 and 1·15 kg. per cow daily

to 4·02 and 0·68 kg., and later to 3·54 and 0·45 kg., over a period of 9 weeks, did not influence the fat content of the milk, but reduced the total solids percentage by an average of 0·5.

The results of a series of experiments in which moderate doses of iodinated protein were given to dairy cows in mid-lactation have been summarized by Bartlett, Rowland & Thompson(27). A greater increase in fat percentage was observed with cows on indoor winter rations than with cows on pasture, the average increases being 0·46 (five experiments) and 0·10 (eight experiments) respectively. The solids-not-fat percentage showed an average increase of 0·04; no effect was observed on the carotene, vitamin A and total aneurin of the milk, but the ascorbic acid, free aneurin and phosphatase showed a marked decrease. Bartlett, Folley, Rowland, Curnow & Simpson(28) have reported the presence of oestrogens in some species of English grasses and clover, and have put forward the suggestion that the rapid increase in milk yield and solids-not-fat content which occurs when cows are turned on to young pasture in the spring may be due, at least in part, to this oestrogenic activity.

Further interesting studies, which are helping to meet the scarcity of information on the genetical determination of the composition of cow's milk, have been reported from Sweden by Hansson & Bonnier(29), Hansson(30), Larsson, Platon, Thomé & Hansson(31), and Hansson, Skjervold & Carli(32). The papers deal with the relation between the fat content and the lactose and protein contents. The relation between fat and solids-not-fat, and the influence of mastitis thereon, have been investigated by Richardson & Folger(33).

Overman & Gaines(34) have re-examined, using three new sets of data, their well-known relation between the caloric value and fat content of milk, in view of the contention of Bonnier, Hansson & Jarl in 1946 (*Acta Agric. suec.* **2**, 159) that the regression was not linear. The regression lines for the first two sets of new data agreed with those of Bonnier *et al.* in being significantly non-linear, but were concave upwards in contrast to theirs which were concave downwards. The third set yielded a strictly linear regression. It is concluded that on the present evidence there is no need to modify the formula.

The increasing interest in the practice of milking cows before parturition has led to further papers(35, 36, 37, 38, 39) on the composition of the colostral-like initial pre-partum secretions, the transition towards normal milk, and the resulting contrast in the composition of the post-partum secretions of pre-milked cows and cows not pre-milked. Parrish and his colleagues, continuing their studies of the properties of normal colostrum, have shown(40) that a high-protein ration increases the non-protein nitrogen content of the colostrum and early milk, and have given data(41) on the general composition.

Milk of other species

The composition of the colostrum and milk of the New Zealand Romney ewe has been investigated by Barnicoat, Logan & Grant(42). Other papers deal with the milk of Karakul sheep in Norway(43) and of Samarkand(44) and Tushinskoy and Gruzinskoy(45) sheep. The effect of the stage of lactation and plane of nutrition on the composition of the milk of Poland China and Chester White sows in America has been described by Bowland, Grummer, Phillips & Bohstedt(46, 47). As regards other species, papers have dealt with some characteristics of the milk of the mare(48, 49), the ass(50) and the elephant(51, 52).

(*b*) *Butter*

Extensive studies, both chemical and microbiological, of first-grade salted butter during storage at 30, 50, 70 and 90° F. in tins and as parchment-wrapped prints have been made by Grant, Gibbons, Marshall & Lips(53). As the flavour scores decreased with increasing time and temperature of storage the free acidity of the whole butter, the acidity of the fat and the serum, and the fluorescence of the serum in light of 365 mμ wave-length, all increased. The pH of the serum decreased with time; the amino-acid titration of the serum increased at first and then decreased. All these changes were greater with the prints than with the tinned samples. The correlations between the chemical and the other tests and scores are discussed.

Jamotte(54) has reported on the iron and copper contents of samples of Belgian butter at different times of the year, and given average values of 0·35 and 0·25 p.p.m. respectively. Keestra(55, 56) has discussed the iron content of Dutch butter and the wide variation from factory to factory and province to province, with an average for the country of 0·65 p.p.m. in 1946. In a subsequent paper Keestra(57) discusses the data obtained by the Netherlands Dairy Control Bureau in 1949 for the iron, copper, moisture and diacetyl contents of Dutch factory butter samples in relation to their quality classification. Ramaswamy & Banerjee(58) have made some observations on the constituents in butter that produce the ghee aroma.

(*c*) *Cheese*

Various aspects of the ripening of Cheddar cheese have been investigated: Harper & Swanson(59) have studied the amino-acids and observed the presence of aspartic acid, glutamic acid, glycine, isoleucine, leucine, lysine, phenylalanine, proline and valine in the unhydrolysed water extract of the cheese as well as in the cheese hydrolysate. Peterson & Johnson(60) have measured the liberation of the very small amounts of the lower fatty acids during making and ripening of Cheddar cheese from raw and pasteurized milk. Sheuring & Tuckey(61) have followed the changes in the constants of the cheese fat, and Calbert & Price(62) in the diacetyl content (and its relation to the flavour) during the ripening of Cheddar cheese. Kosikowsky & Dahlberg(63) have estimated the tyramine contents of Cheddar cheese of varying ages and of a number of cheese of other well-known types. Tyramine was found in all the samples, and in much higher concentration than was anticipated from the meagre literature available. The patchy discoloration of anatto-coloured cheese has been investigated by Barnicoat(64), who, on the basis of his experimental observations, suggests that the mechanism involves the production of sulphydryl compounds which, in the presence of air entering through cracks in the rind, promote oxidation of the unsaturated groups of the carotenoid bixin of the annatto and thus cause its bleaching.

The ripening of Edam cheese, particularly the influence of varying pH and temperature on the protein breakdown, has been studied by Raadsveld & Mulder(65), and the proteolysis of Emmenthal cheese by Storgårds & Hietaranta(66). The latter found that the development of flavour was associated with increasing glutamic and aspartic acid contents, glutamic acid being mainly responsible for the pleasant flavour of mature Emmenthal. Schwarz & Mumm(67) have followed the conversion of lactose to lactic acid during the manufacture and early ripening of Tilsit cheese, and have also(68) investigated the effect of the heating of the milk, by partially denaturing the soluble proteins and rendering them precipitable, on the composition of the whey and cheese.

The small crystals which form in some samples of processed cheese have been shown by two investigators, Leather(69) in England and Blanchard(70) in Canada, to consist of calcium tartrate. The presence of well-developed facets and central inclusions in the small crystals (of size about $0{\cdot}9 \times 0{\cdot}9 \times 2$ mm.) suggests that they grow slowly *in situ*(69), presumably from tartrate added as an emulsifier reacting with the calcium of the cheese.

(*d*) *Dried and condensed milk*

In collaborative work on the deterioration of dried skim milk during storage, Lea & White(71) have made a valuable contribution to the knowledge of the chemical, physical and palatability changes involved. Three spray-dried separated milk powders with moisture contents of 3·0, 5·0 and 7·6% were packed in air, and in almost pure nitrogen, in gas-tight cans and stored at 20·0, 28·5 and 37° C., for a period of nearly 2 years. The powders were examined at intervals for palatability, colour, pH, equilibrium relative humidity (indicating crystallization of the lactose), conversion of β-lactose to α-lactose hydrate, decrease in total soluble lactose, absorption of oxygen, production of carbon dioxide, solubility in water at 20 and at 50 or 60° C., changes in the distribution of soluble nitrogen, reducing power towards potassium ferricyanide, base-binding capacity, formol titration, weight of the undialysable fraction, free amino-nitrogen by the Van Slyke method, sugar attached to the protein, and bacterial content.

Little change was observed in the powders of low and medium moisture content, except in

palatability and gas exchange at the higher temperatures. The powder of the highest moisture content, particularly at the higher storage temperatures, rapidly became unpalatable, discoloured and insoluble. Its pH, free amino-nitrogen, and soluble lactose content fell; the amount of sugar attached to the protein and the reducing power of the powder towards potassium ferricyanide increased. Oxygen was absorbed and carbon dioxide produced. It was suggested that a major cause of deterioration in powder of high moisture content, particularly at high storage temperatures, is a reaction involving the free amino-groups of the milk protein, which will consist very largely of the ϵ-amino-groups of the lysine residues. The first stage of this reaction appears to be between the protein amino-groups and the potential aldehyde group of reducing sugar. The rate of the protein-sugar reaction is largely determined by the moisture content or, more correctly, by the activity of moisture in the powder, and is very much greater at 7·6 than at 5·0 or 3·0 %.

The composition of the soluble and insoluble portions of reconstituted milk powders have been explored by Waite & White(72). Samples of dried whole and skim milk were reconstituted at 20 and 50° C. and centrifuged; with samples of low solubility protein was found in the fat layer and fat in the sediment. The washed sediment consisted of denatured casein, along with calcium and phosphorus in the proportions in which they are found in tricalcium phosphate. When dried skim milk of various moisture contents was stored at a range of temperatures, Borrell(73) observed that appreciable amounts of lactose were destroyed at 7·2 % moisture content and a temperature of 37° C., but negligible amounts at 2·7 % and 20° C. The conversion of β-lactose into the α-form also occurred more rapidly at the higher moisture contents and temperatures.

Deysher & Webb(74) have analysed the crystalline deposits from cans of commercial evaporated milk and shown that the great majority of the crystals contained 98 % tricalcium citrate with small, but varying, amounts of tricalcium or trimagnesium phosphates. One type of deposit, however, contained at least 10 % of tricalcium phosphate. In studies of heated milk Patton(75) observed the formation of 5-hydroxymethyl-2-furfurol when condensed skim milk, or a solution of β-lactose and glycine, or of lactose and sodium caseinate, were autoclaved at 127° C. for 2½ hr.; and Keeney, Patton & Josephson(76) identified acetol and acetic acid among the products of heating condensed skim milk at 122° C. for 3 hr.

II. Chemistry of milk constituents

(*a*) *Milk fat*

The properties of New Zealand butterfats, as reflected by their iodine, Reichert and saponification values and softening points, have been investigated by Cox & McDowall(77) on monthly samples from nine commercial factories over a period of 4 years. A remarkable uniformity of values was found between the factories and in the different years. Over the 4 years the minimum and maximum values of the monthly average, and the grand average, were: 33·8, 40·2 and 36·7 for the iodine value; 25·5, 32·3 and 30·4 for the Reichert value; 222·5, 232·7 and 229·5 for the saponification value; and 32·2, 33·7 and 33·1° C. for the softening point. The iodine values, surprisingly, were lowest in November and December and highest in June.

Jarl(78) has given an interesting paper summarizing his researches on the influence of different nutrients, and of changes in body weight, on the iodine value of the butterfat produced. He found no correlation between the iodine value of the butterfat and the iodine value of the fat in the cow's food, or between the iodine value of the butterfat and body-weight gains, but with the extent of body-weight losses there was a significant correlation. In a feeding experiment the addition of excessive digestible protein, in the form of dried skim milk, to the cow's diet significantly decreased the iodine value of the butterfat—suggesting that the high protein content of the herbage is not responsible for the high iodine value of the butterfat when cows are on spring pasture. Frye, Cannon & Bird(79), in a feeding experiment comparing cracked soya beans and linseed meal as the protein supplements, found higher iodine values for the butterfat when the linseed meal was used. They also observed that a significant correlation existed between the

mean barn temperature and the degree of unsaturation of the butterfat, particularly the temperature recorded one day before the milk samples were taken. Wauschkuhn(80) has discussed the refractive index, and its relation to the hardness, of butterfat in the Hanover district.

Anantakrishnan, Bhalerao & Paul(81) have studied the effect on the glyceride composition of the milk fat of giving Sindhi cows supplements of 1·5 lb. of various oils daily. The unsaturated cottonseed, sesame and hydrogenated groundnut oils increased the iodine, refractometer and saponification values while reducing the Reichert, Polenske and Kirschner values. The relatively saturated hydrogenated coconut oil decreased the Reichert and Kirschner values but had little effect on the other constants. The result of the application of the ester fractionation method to the milk fats is also given. Mattsson(82) has investigated the poly-unsaturated fatty acids in butterfat. After alkali isomerization, butterfat showed absorption maxima at 232 and 268 mμ corresponding to linoleic and linolenic acids; but before the isomerization a maximum was observed at 232 mμ due to a C_{18} unsaturated fatty acid containing two conjugated double bonds. This acid is present in the milk fat and remains unchanged during churning. When the cows were given a diet including linseed oil, which has no conjugated C_{18} unsaturated acid, the amount of this acid rose from 0·5 to 6·7 % of the butterfat, but the linoleic and linolenic acid content did not change appreciably.

The fractionation of milk fat by molecular distillation has been reported by Jack & Olsen(83) who removed seven fractions at intervals between 140 and 190° C. The iodine values increased and the saponification values decreased with successive fractions. Sadgopal(84) has examined the butterfat from the milks of various species—cow, buffalo, goat, sheep, camel, mare, ass, elephant and sow—for iodine, saponification, Reichert, Kirschner, Polenske, and 'A' and 'B' values. He has compared his results with those of earlier workers, and classified the fats according to their characteristics. Paul & Anantakrishnan(85) have recorded the constants of the colostral fat of goats and sheep, and with their colleagues(86) the composition of the free fatty acids in high acid ghee.

(*b*) *Nitrogenous constituents and sugars*

As already described (p. 229), Lea & White(71) showed that a progressive loss of free amino-nitrogen and an increase in combined sugar occur in the undialysable (protein) fraction of skim-milk powder of high moisture content during storage. This suggested that a reaction of the Maillard type between amino-acids and reducing sugars was responsible for part at least of the undesirable changes which develop in milk powder under such conditions. Lea(87) has tested this point by storing 'dry' dialysed milk protein for 6 months at 37° C. in the presence and absence of sugar, noting the effects of lactose in the relative proportion normal to milk, of glucose at the low concentration reported in milk, of sucrose, and of mixtures of these. The reducing sugars combined with the free amino-groups of the protein, and in a 1 : 1 ratio, but sucrose did not. Glucose reacted more rapidly with the protein than did lactose, and the complex formed became discoloured and insoluble much more rapidly. For the determination, in these studies, of the free amino-nitrogen of the protein Lea(88) utilized his procedure based on the reaction with nitrous acid in the manometric Van Syke apparatus.

Neseni & Körprich(89) have dealt with the variations in the non-protein nitrogen content of the milk of individual cows with stage of lactation and season of the year; and Block & Bolling(90) have listed the amounts of various amino-acids that they have estimated in the non-protein nitrogen fractions of human milk, human colostrum and cow's milk.

The presence of a small amount of glucose in milk was reported, but not fully confirmed, some twenty years ago. Recently, Anantakrishnan & Herrington(91) have definitely demonstrated the presence of from 4·08 to 7·58 mg. of glucose per 100 ml. of cow's milk, and of up to 15 mg. per 100 ml. of colostrum. This was done by selective fermentation and chromatographic methods applied to deproteinized and deionized milk. Leviton(92) has developed a method for the extraction of lactose and soluble proteins from skim milk powder with methanol. The addition of methanol at −15° C. to spray-process products containing lactose results in the formation of

a supersaturated solution of lactose, and there is sufficient time between protein precipitation and the start of lactose crystallization to permit a separation. The purity of the lactose thus obtained by a single crystallization compares favourably with the refined lactose of commerce. The proteins are readily dispersable in water and undergo little alteration in composition. The effects of methanol concentration, temperature, and the ratio of solvent to powder are discussed.

(*c*) *Miscellaneous*

The cholesterol, and the associated butterfat, contents of the milk of Holstein, Jersey and Guernsey cows, on winter feed and on pasture, have been investigated by Nataf, Mickelsen, Keys & Petersen (93). Mean values of 11·4 and 11·3 mg. of cholesterol per 100 ml. of milk, ranging from about 7 to 17, were obtained for the two regimes, with no significant breed differences in summer but lower values for the Holstein milk in winter. There was a close correlation between the cholesterol and fat contents of the milk samples. The phospholipid contents of milk, cream, butter and buttermilk have been compared by Mohr (94).

A range of 0·122–0·258 (mean 0·18) % citric acid in the milk of individual cows was found by Storgåards (95), who records the distribution of the acid into separated milk, cream and separator slime, and the effect on it of pasteurization and cheesemaking. Herreid & Francis (96) have investigated the change in the oxygen content of milk during production and processing. Archibald (97) has measured the nickel content of cow's milk, eventually finding that nickel was not present when the cows were hand-milked into glass vessels.

(*d*) *Enzymes*

Phosphatase

Interest has been well maintained in the enzyme, destruction of which forms the basis of the principal method of assessing the efficiency of milk pasteurization. In a survey presented to the XIIth International Dairy Congress, Kay, Aschaffenburg & Mullen (98) drew attention to the serious lack of precise data on the destruction of phosphatase under H.T.S.T. conditions of heating. Provision of such data depends primarily on the design of apparatus allowing accurate and even heating of milk for short periods at relatively high temperatures. This has been attempted in various ways (99, 100, 101, 102, 103) to a varying degree of precision. Of the data on the thermal destruction of phosphatase, the most accurate appear to be those obtained by Danish workers (99) who used an unconventional, but efficient, technique of heating. In this, heat-inactivated skim milk is held in ampoules at a temperature slightly above that required for pasteurization, and a predetermined volume of buttermilk which contains phosphatase in high concentration is added so that the mixture attains the desired temperature. By plunging the ampoules into ice-water after the desired holding time, heating for precise time-temperature combinations is achieved. A thorough study of the Danish report is a necessity for all those interested in this problem. Details of a promising type of laboratory pasteurizer have been given by Lindqvist (104), but no results on phosphatase destruction have so far been published. Equipment designed to simulate H.T.S.T. plant conditions has been described by Thomé & Olsson (105) and by Pascoe (106).

Studies of the distribution of raw-milk phosphatase between cream and skim milk have come from Hetrick & Tracy (102), and from Hansson, Solberg & Sjöström (107), who observed unusually strong enzyme action on high dilution of cream. From comparative tests with various enzyme inhibitors, Zittle & Della Monica (108) concluded that milk phosphatase bears a closer relationship to bone and kidney than to intestinal phosphatase. Extensive data on bacteria which produce phosphatase at pH 10, and on the greater heat resistance of the bacterial enzyme as a means of its differentiation from milk phosphatase, have been given by Tittsler, Sager & Sanders (109).

A simple and rapid phosphatase test using *p*-nitrophenylphosphate as the substrate has been proposed by Aschaffenburg & Mullen (110). It has been found satisfactory by Ludlam & Hemingway (111) who applied it to 1000 commercially pasteurized milk samples which were also subjected to the Kay-Graham test. Promising results were also obtained by Sjöström & Liliebladh (112) who studied its possible use as a rapid field test. The method has been improved

and simplified by Tramer & Wight(113), who have introduced permanent colour standards so chosen that the results correspond closely to those obtained in the official Kay-Graham test. Kosikowsky(114) has modified conventional phosphatase methods to obtain a procedure applicable to a variety of liquid and solid dairy products. In collaboration with Dahlberg(115, 116), he has developed for testing cheese a technique characterized by the extraction of the free phenol with ether. This step eliminates the danger of interference by substances like tyrosine, tryptophane and tyramine, these being insoluble in ether.

Horwitz, Knudson & Weiss(117) have presented the results of a collaborative study on the comparative value of various phosphatase tests used in the United States. The methods of Gilcreas & Davis and of Sanders & Sager gave reliable results with milk. The latter had the advantage of remaining applicable to testing cheese. Sanders & Sager(118) have described suitable preservatives for samples to be subjected to their modification of the phosphatase test. The possibilities of using this modification under Australian conditions of pasteurization have been discussed by Smith & Vincent(119).

Swedish work concerning milk phosphatase has been summarized by Sjöström(120). Awakening interest in the phosphatase test has come from countries in which the test had hitherto received little attention. Evidence for this can be found in reports from Switzerland(121, 122, 123, 124) and Germany(125, 126) where Schwarz & Fischer(127) have studied and modified the Scharer technique.

High phosphatase readings, and their relation to the degree of overheating of sterilized milk, have been discussed by Clegg & Lomax(128). Kannan & Babu(129) have studied the effect of the state of lactation and season on the phosphatase concentration in the raw milk of Indian cows, buffaloes, goats and sheep. They have also determined(130) the optimum pH of hydrolysis, and have measured activation by various ions and amino-acids of the phosphatases present in the milk of these species. They found evidence for the presence of an acid phosphatase in the milk of the goat and the sheep, but not in that of the cow or buffalo. This is surprising, since Mullen(131) has shown that cow's milk contains small, but definite, amounts of an acid phosphatase whose properties he has studied in detail. It is much more heat resistant than the alkaline phosphatase.

Rennin

A critical review of the properties of rennin, and of current views and theories on the milk-clotting mechanism, has been provided by Berridge(132). Part of a broader study of the physical chemistry of milk by Kopaczewski deals in detail with the problem of rennet coagulation(133). The effects of pasteurization on curdling and the properties of curd have been investigated by Van der Waarden(134), with particular reference to the casein complex. Acid precipitation in casein solutions that had been subjected to rennet, pepsin or trypsin action at various pH values has been studied by Gonashvili(135). Van Dam's method of assessing the relative strength of rennet extracts has been critically examined by Mulder & Radema(136). By alcohol precipitation, Kopaczewski(137) obtained from rennet extracts a component that appears to counteract rennin action. Sattler(138) has reported on the presence of substances capable of promoting or inhibiting bacterial growth in certain aqueous rennet extracts. According to Zollikofer(139), homogenization of milk increases the speed of clotting by rennet, and reduces the fat content of the resulting whey. The effect on cheese yield of adding varying amounts of calcium chloride to milk, has been studied by Orsenigo(140); optimal yields were obtained when about 5 g. per 100 l. milk were added. Schwarz & Mumm(141) have reported that the quality of Tilsit cheese did not suffer when 10 g. of calcium chloride were added per 100 l. of milk, an addition that allowed a reduction in the quantity of rennet of about 5 %. Addition of sodium nitrate was ineffective, and that of potassium nitrate deleterious. For Cheddar cheese-making, Babel(142) has advised against calcium chloride addition to save rennet, because it reduces protein degradation, and produces cheese of poor body and texture. Monthly tests of clotting time and curd firmness for the milk of some 500 individual cows have been reported by Peltola(143) who studied the relation of these characteristics to the individuality of the cow, season, feeding, milk yield and other factors.

Indian workers have described the preparation of vegetable rennets from *Ficus carica* (144) and *Streblus asper* (145). The rennet from *Ficus carica* proved the superior in making Cheddar cheese.

Other enzymes

The time-temperature combinations required to inactivate milk lipase have been determined by Hetrick & Tracy (146); a straight line resulted when temperature was plotted against the logarithm of the time of heating. In continuing their lipase studies, Johnson & Gould (147) have been mainly concerned in improving analytical procedures, and have given particular attention to the solvent extraction method for the recovery of fatty acids from cream. Peterson, Johnson & Price have described a method for determining lipase in cheese (148), and have studied lipase action in cheese made from raw and pasteurized milk (149).

A kinetic study of the formation of coloured complexes between the iron atom of the peroxidase molecule and hydrogen peroxide has been presented by Chance (150). Data for the relative peroxidase content of the milk of various species have been given by Rumanian workers (151). According to Vorstman (152), the Storch test is best carried out after adjusting the pH of milk to about 7·2. Roth (153) has studied the inhibiting effects exerted by *p*-aminobenzoic acid and a number of sulphonamides on the action of peroxidase and of Schardinger's enzyme.

French workers (154, 155) have continued their work on the absorption of xanthine oxidase on the fat globules of milk and its release into the aqueous phase as induced by physical changes or chemical action. Polarography has been used by Knobloch (156) to study the activity of the xanthine oxidase and aldehyde oxidase in milk and in milk fractions. Kalckar & Klenow (157) have described methods for measuring the activity of xanthopterin oxidase in preparations obtained from whey by ammonium sulphate fractionation. The proteinase of cheese and its properties has been studied by Peterson, Johnson & Price (158) who have worked out a method for determining the activity of this enzyme. Data on the heat inactivation of most of the milk enzymes can be found in a paper by Hájek (159).

III. Physical chemistry

Proteins

An authoritative review of present-day knowledge of the physical chemistry of the milk proteins has been provided by McMeekin & Polis (160). Ballarin (161) has discussed the various interrelated physico-chemical equilibria existing in milk with particular regard to those involving the proteins.

Casein

Differences in the amino-acid composition (162, 163), specific volume (164) and rennet coagulation (165) of α- and β-casein have been reported. Ter Horst (166) has presented a well-documented critical review of current concepts of the calcium caseinate-calcium phosphate complex. Hostettler, Rychener & Künzle (167) subjected five dispersions, obtained from milk by stepwise centrifugation, to analysis by physical and chemical methods. From the absence of streaming birefringence it was concluded that the casein of milk exists in the form of spherical, optically anisotropic particles. Calcium and phosphorus analyses suggested a preferential association of these elements with the coarser particles. That the colloidal casein particles in milk are spheres or spherical aggregates was also concluded by Ford & Ramsdell (168) who, like Hostettler *et al.*, but by a different technique, removed these particles gradually by centrifugation. According to Ford & Ramsdell, the colloidal casein occurs in two size ranges, the sizes in each range being limited in number and forming multiples of one unit size.

Preliminary results of a polarographic study of the casein complex have been given by Pyne (169). As calcium salts are unsuitable for polarography, he replaces the calcium in the complex by barium. Chevalier, Mocquot, Alais & Bonnat (170) have experimented with milks varying widely in their rennet clotting time. Their results suggest that the variations are bound up with differences in the make-up of the casein molecule or its complex. The caseinate obtained

by supercentrifuging from slow-clotting milk was found(171) to have a lower calcium-nitrogen ratio, a smaller particle size, and a greater degree of hydration than that of fast-clotting milk. The properties of casein gels formed in acid or alkaline solutions with or without the addition of alcohols have been investigated by Magis(172). Mellon, Korn & Hoover(173) have continued their studies of the water absorption of casein and benzoylated casein. The reaction of casein with formaldehyde has been the subject of further study(174).

The antigenic properties of various fractions and compounds of casein have been examined by Kesztyüs & Bagdy(175), and the effect of casein additions on the heat inactivation of the antibody to pneumococcal polysaccharide has occupied the attention of Krejci, Jennings & Smith(176). Contributions to the knowledge of human milk casein have come from Mellander(177) and Derrien, Liardet & Roche(178). Guidance in the industrial preparation of acid and rennet casein has been given by De Vleeschauwer & Hendrickx(179).

β-Lactoglobulin

Using the X-ray diffraction technique, Senti & Warner(180) have determined the molecular weight of orthorhombic crystals of β-lactoglobulin, wet with mother liquor, to 35,400. Cecil & Ogston(181) have arrived at exactly the same value from determination of the sedimentation and diffusion constants. A slightly lower value of 33,700 has been reported by Halwer & Brice(182) who employed Debye's light-scattering method, and a markedly higher one of 39,590 by Christensen(183) who worked with a micro-osmometer.

There is increasing evidence that β-lactoglobulin, though crystalline, is not homogeneous. Thus, heterogeneity has been revealed by electrophoretic examinations(184, 185), by the use of the method of electrophoresis-convection(186), and even by immunological methods(187).

Stein & Moore(188) have given chromatographic results for the amino-acid composition of β-lactoglobulin which, on the whole, agree well with those determined by other methods. Klotz(189) has shown that, of a number of crystalline proteins examined, only β-lactoglobulin was able to bind methyl-orange in appreciable quantities. McMeekin, Polis, Della Monica & Custer(190) have prepared a crystalline compound of β-lactoglobulin with two equivalents of dodecylsulphate, and have compared its properties with those of the protein itself. When lactoglobulin and some other proteins are heated to 100° C. at pH 9, an intensified colour reaction with Folin's phenol reagent, and with diazobenzene sulphonic acid, results(191). The authors explain this by the removal of steric hindrance occurring when the tightly folded peptide chains of the native proteins are opened up by denaturation.

Whey proteins

Several studies concerning the separation and characterization of the 'whey' proteins of milk have been reported. That bovine whey contains three, if not more, components other than β-lactoglobulin has been revealed by the electrophoretic data of McMeekin, Della Monica & Custer(192). Six components were found by Stanley, Andrews & Whitnah(193) on electrophoretic examination of the proteins prepared, either by straight dialysis and lyophilization or by dialysis after acid precipitation, from the whey obtained by saturation of skim milk with sodium chloride. Though six components were present after each treatment, their quantities differed. Bain & Deutsch(194) have outlined a scheme for the isolation of the whey protein fraction of cow's and goat's milk using the low-temperature alcohol fractionation technique. It is noteworthy that none of the isolated fractions could be obtained in the crystalline state. In a solubility study of the soluble proteins of human milk, Derrien, Liardet & Roche(195) found evidence for the presence of at least ten components.

Immune proteins

The increased interest in the immune globulins of colostrum, commented on in the previous review, has been well maintained. E. L. Smith, who has himself contributed notably to our knowledge of these proteins, has reviewed the whole subject at length(196). A concise discussion

of the problem of the transfer of immunity from colostrum to the calf(197) is also well worth reading. The technique of electrophoresis has been of great value in demonstrating the appearance of new globulin components in the serum of the newborn calf(198, 199) and lamb(200, 201) following the ingestion of colostrum. For the bovine, Smith & Holm(199) have established the identity of this component with the immune globulin of colostrum. The fraction differs slightly, but significantly from the γ- and T-components. That heterologous transfer is possible has been demonstrated by Hansen & Phillips(202) who gave cow's and pig's colostrum to newborn kids. Aschaffenburg(203) has described a simple and rapid serum turbidity test by which the transfer of immune globulins from as little as 200 ml. of colostrum can be demonstrated. That the proteins reach the bloodstream of the calf by way of the lymphatic system after absorption from the small intestine, has recently been shown by Comline, Roberts & Titchen(204). Further data on the amino-acid composition of colostral immune globulins have been given(205, 206). Bovine and human colostrum have been found to contain a trypsin inhibitor(207) which might be of importance in keeping ingested antibodies intact.

Other aspects of physical chemistry

Kopaczewski(208, 209) has presented a lengthy literary and experimental study of the physical chemistry of milk dealing, in particular, with coagulation and dispersion problems.

Surface activity, foaming

Whitnah, Conrad & Cook(210) have given a detailed description of a vibrating jet apparatus suitable for measuring the surface tension of freshly formed surfaces of milk, milk filtrates and milk products. Extremely rapid falls in surface tension were observed, the level of static values being attained in fractions of a second. Messeri & Piccardi(211) have observed that the surface tension of human milk increases by about 10 % during the first fifteen minutes after its withdrawal. Nelson(212) has related surface tension to colour and grain formation in evaporated milk. The marked effects on surface tension of adding small amounts of milk to alkaline detergents have been studied by MacWalter & Tyrrell(213).

Richardson & El-Rafey(214) have continued their studies of the foaming properties of milk and milk products by dealing with the role of the milk lipoids. Apart from studying, at various temperatures, the foaming capacity and foam stability of systems containing skim milk and increasing amounts of butterfat, they report on the foaming behaviour of a diversity of artificial emulsions. Maass(215) believes that milk contains a special heat-stable 'Schaumstoff', because firm and stable foams could be produced from whey prepared from milk after heat denaturation of the soluble proteins.

Heat treatment

The complexities of interpreting data about the effects of heat treatment on the reducing systems in milk and milk products have been well brought out by workers from Minnesota(216, 217) who have shown that the measurements differ widely according to the method of testing. Thus assessment of the reduction of aneurin disulphide or nitroprusside reflects largely the groups formed through heat changes in the whey proteins, whilst assessment with acid ferricyanide(395) measures in addition reduction by ascorbic acid and groups formed in heated lactose. The iodosobenzoate method again reflects the activity of different reducing groups, as shown by experiments on the effects of heat on buffered solutions of dialysed whey(217). The aneurin disulphide and acid ferricyanide methods were used to study the changes caused by various heat-treatments in the reducing capacity of spray-dried whole milk, and of skim-milk powder reconstituted to different solid contents(216). Using the dichlorophenol-indophenol method of determining ascorbic acid, Tammisto(218) has assessed the reducing groups formed by holding milk in a pasteurizing plant for 3 or 20 sec. at temperatures from 80 to 92° C. Their formation increased with time and temperature of treatment. The author has followed the fate of the reducing groups in souring and churning, and during the storage of buttermilk. Sjöström &

Larsson(219) have applied a modified ferricyanide technique to samples of skim milk, whole milk and cream held for 25 sec. at temperatures ranging from 72 to 92° C. in a laboratory pasteurizer. In general, the reducing power increased with the temperature of heating. Patton(220) has discussed the mechanism of maltol formation during prolonged exposure of skim milk to high temperatures. He and Josephson(221) have described a method of isolating furfuryl alcohol from heated skim milk; unheated milk does not contain the alcohol. Vodak & Tarassuk(222) have determined the changes in buffering power occurring when milk was held for 24 hr at 4·5° C. after it had been heated for 30 min. at temperatures ranging from 42·5 to 93° C. The buffering power decreased with the temperature of heating, the changes proving to be reversible to a large extent during subsequent storage at 4·5° C.

Freezing

With the realization of the value of preservation at very low temperatures, knowledge of the physical and chemical changes caused by freezing and frozen storage has become of increasing importance. Following two earlier papers, Babcock and his colleagues have presented a series of further studies(223, 224, 225, 226, 227) concerning frozen homogenized milk. Further data are given on the uneven distribution of solids after stationary freezing(223), and on the even distribution following slow rotation of the milk during freezing(227). In another paper of the series(226), it is reported that the addition of 2 g. of sodium citrate per l. of homogenized milk proved effective in preventing the protein flocculation normally observed on thawing the milk after prolonged storage at −17·8° C. Ascorbic acid additions were ineffective in this respect though of possible value in retarding flavour defects. By adding both citrate and ascorbic acid, the keeping life could be doubled without loss in appearance or flavour. The beneficial effect of ascorbic acid addition on the prevention of flavour defects has also been demonstrated by Anderson, Betzold & Carr(228), and by Bell(229) working with holderpasteurized homogenized milk kept frozen in sealed containers. Bell has measured the changes in the oxidation-reduction potential occurring at different temperatures of storage. Elsewhere, Bell & Mucha(230) have shown that oxidative changes in raw or pasteurized milk, or in mixtures of the two, held at −17° C. can be more successfully retarded by the addition of ascorbic acid than by oxidation of the native ascorbic acid with hydrogen peroxide.

Interesting results have been provided by Davidov(231). In contrast to milk frozen in bulk, milk frozen in layers or agitated during freezing at −18° C. was found on thawing to be free from flaking due to protein flocculation, and from oiling off of the fat. When milk frozen in layers was kept at different temperatures, flaking decreased with the storage temperature. It was absent after storage for 18 months at −25° C. Davidov suggests that freezing may cause an accumulation of salts and acid in some of the bound water and prevent its freezing. Flocculation of proteins may be the result of contact with this bound water solution. In storage experiments at temperatures ranging from −5 to −25° C., it was shown that flaking decreased with the temperature of storage. It still occurred at −18° C., but was absent after storage at −25° C. The bound water content fell from 11 to 8 % when milk was kept at −5·7° C. for two months; no change occurred with milk stored at −25° C.

The results of comparative trials of the keeping properties at −10° F. of fresh milk, and of condensed and dried milk made from it, have been reported by Tracy, Hetrick & Krienke(232). Colvard & Roberts(233) found no evidence of protein or flavour deterioration after storage, for 20 weeks at −12° F., of milk concentrated to one-third of its original volume. Storage at 0 or 10° F. proved inadequate to prevent these faults. According to Savinosvsky(234), use of very low temperatures is also essential in prolonged storage of sour cream. Keeping at −25° C. proved satisfactory whereas storage at −10 or −15° C. led to flaking and oiling off. No appreciable acidity developed at these temperatures. A report from Barnicoat(235) deals with butter storage at various temperatures between 60 and −5° F. Low temperatures improved the keeping life, but the advantages of storing butter at −5° F. rather than at 14° F. were too small to warrant the extra refrigeration costs.

Discrepant results are revealed by reports dealing with the storage of cheese at relatively low temperatures. Russian workers (236, 237) found prolonged storage of several types of hard cheese satisfactory at −5° C., but unsatisfactory at 1–2° C. Ryutov (236) has pointed out that the lower temperature is just above the freezing-points of the samples, which he determined as −6·8 to −10·3° C. for mature Dutch cheese, −7·8 to −10·1° C. for mature Soviet cheese, and −6·3 to −6·5° C. for semi-mature Backstein cheese. Extensive work from Sweden (238), on the other hand, shows that Gouda, Svecia and Manor cheese could with advantage be kept at 2° C., the temperature found to be unsatisfactory by the Russian workers. Analytical data, including the results of determinations of the total and soluble nitrogen content of the products, are given in all the reports.

Miscellaneous

Little has been added to our knowledge of the membrane surrounding the fat globules. The results of the first attempts to extend this knowledge by the use of the electron microscope (239, 240) must be considered disappointing. Further experience, particularly concerning the difficult preparation of specimens, is clearly needed before findings can be interpreted with confidence. Based on a study of the fat-globule membrane and of the foaming properties of cream, Belousov (241) has given a physico-chemical theory of the churning process.

Hansson (242) has observed that addition of phosphatides or blood serum globulins enhance the creaming power of milk. Data on the rate of creaming of raw cow's and buffalo's milk have been given by Fahmi (243). Factors that influence 'cream line' in practice have been discussed by Mohr & Kaufmann (244).

Rangappa (245, 246) has continued his investigations of the refractive index of cow's and buffalo's milk. Refractivity data on ghee have been given by Achaya & Banerjee (247). According to Mikhaylov (248), the specific heat rises linearly with temperature for dried skim milk between −11·8 and 35° C., and for liquid milk fat between 30 and 74° C. Schwarz & Mumm (249) have studied the effect of holding fresh milk for 24–48 hr. at 5° C. on clotting time, and on the ultrafiltrable calcium, phosphorus and nitrogen of the milk and rennet whey. Buffalo's milk has been found to contain more total and colloidal calcium than cow's milk (250) with a higher precentage of the total in the colloidal form.

Dutch authors (251) have described a large-scale process for the reduction of the salt content of whey concentrates by electrodialysis, and have discussed (252) the nutritional merits of the resulting products. According to Nelson (253), the heat-induced colour changes occurring during the preparation of evaporated milk can be suitably assessed by reflectance measurements in a Beckmann spectrophotometer. The darkening effect of different sterilizing temperatures has been measured by this method (254). Ashworth & Hibbs (255) have shown that the ease of dispersion of dried whole milk and whey depends on the degree to which the liquids are condensed prior to drying.

The first two parts of a book by Mulder deal, from the physico-chemical viewpoint, with problems concerning the fat in milk (256) and concerning the churning process and the properties of butterfat (257). Van Kreveld (258) has given a useful description of a microscopical technique for determining the size-frequency distribution of the fat particles in milk, evaporated milk and homogenized evaporated milk. In further papers (259, 260) he has elaborated on the observation that the distributions were more regular than those expected from statistical random distribution calculations. Sandelin (261, 262) has determined size-frequency distributions for homogenized milks, and has estimated that the phosphatide layer surrounding the fat globules is of molecular dimensions. Andersen (263) reports on the degree of fat solidification in cream cooled in a variety of manners. A full description of his dilatometric equipment is given. Using a calorimetric technique, Brunner & Jack (264) have measured the degree of fat solidification in creams cooled in various ways, and have shown that churning time depends not only on this degree, but also, possibly, on the distribution of the liquid and solid fat within the fat globules.

Valuable contributions to our knowledge of the physical state of the fat in butter prepared by

the conventional method and by continuous processes have been made by King(265, 266, 267, 268) and King & Fritz(269). Using King's method, Mohr & Baur(270) determined the percentage of globular fat in samples of conventional, Alfa and Fritz butter, with results that did not agree fully with those reported by King(265). Under the microscope, the fat globules in cream containing 80 % fat show considerable deformation. The possible effects of this deformation on the making of Alfa butter have been discussed by Mohr & Wellm(271).

IV. Defects of milk and milk products

(*a*) *Adulteration and its detection*

Added water

The freezing-point test. In this country, considerable attention has been given to some of the finer points of technique of the Hortvet method. By experiments in which a platinum resistance thermometer replaced the usual Hortvet thermometer, Aschaffenburg & Hall(272) brought out the difficulties so often encountered when concentrated sucrose solutions, e.g. 10 %, are used in standardizing Hortvet thermometers. They recommended that such thermometers should be standardized by a recognized testing institution, such as the National Physical Laboratory. Their recommendation was criticized by Sutton & Markland(273) who stated that its use led to lower values for the freezing-point depression of sucrose solutions than those obtained by the customary procedure. This statement was not confirmed by a collaborative experiment(274) in which six laboratories, following agreed details of technique, used the procedure recommended by Aschaffenburg & Hall. In another paper, Sutton and his colleagues(275) have discussed the Hortvet technique and possible sources of error in great detail.

Extensive data on the freezing-point of commercial bulk milks have been provided by Macdonald(276) in this country; the depressions varied but little from an annual mean of 0·544° C. and no seasonal trends were discernible. Contrary to this, Rees(277) reports to have found wide variations in the freezing-point depression of Tasmanian bulk milk. Values as low as 0·506° C. were recorded. It is hard to believe that these refer to genuine milk. Marked seasonal trends were noted, and these followed closely those for the solids-not-fat content. As these findings contradict the general experience of an absence of large seasonal variations and of a lack of correlation with solids-not-fat, confirmation based on samples which are undoubtedly genuine is to be hoped for. Information on genuine milk of Indian breeds of cattle (mean 0·548° C.) and of buffaloes (mean 0·549° C.) has been given by Dastur(278). Studies of the freezing-point of cream(279) and reconstituted skim milk powder(280) have been reported.

Hillman, Provan & Steane(281), and Aschaffenburg & Rowland(282), have described cases of genuine milk with abnormally low freezing-point depressions. The two cases had many features in common. Both occurred under conditions of winter feeding in large herds which had no access to drinking water when housed at night. Depressions below 0·530° C. were frequently found in evening milk whereas in morning milk the values were above normal. The resulting unusually large morning-night differences (e.g. 0·525–0·560° C.) were accompanied by correspondingly large differences in the solids-not-fat (e.g. 8·26–8·83 %) and its constituents. The unbalanced water intake of the cows due to deprivation at night has been suggested(282) as the possible cause of these anomalies. Publication of the first of these reports(281) was followed by a lively correspondence(283, 284, 285, 286, 287).

Other methods. Tests for the detection of added water based on the determination of the refractive index of the usual milk sera, continue to exert a peculiar fascination, although there is ample evidence of their failure except in cases of gross adulteration. Further work(288, 289, 290, 291, 292, 293, 294) has done nothing to change this view. Goded y Mur(295) has described a scheme in which no less than forty characteristics of milk are determined to detect watering or fat-abstraction. It does not include the freezing-point test. When it became impracticable to use this test owing to post-war conditions in Austria, Tellmann & Daim(296) investigated the possibility of calculating freezing-point values from the results of chloride and

refractive index determinations. The general agreement between calculated and determined freezing-points was good, but this did not hold in a number of individual cases.

Other types of adulteration

Methods for the detection of cow's milk in human milk have been reviewed by Rössler (297) who describes a serological test sensitive to the presence of 5 % of cow's milk. Chemical methods which are also suitable for the detection of goat's and sheep's milk in human milk have been given by Romeyer (298, 299). The characteristic smell of goat's milk obtained when milk is distilled in the presence of sulphuric acid, forms the basis of a test for the detection of the adulteration of cow's milk proposed by Vymetal (300). Sastry & Dastur (301) have experimented with various methods in attempts to detect the presence of reconstituted milk powder in whole milk.

(b) Flavour defects of milk and milk products

Oxidative defects, their causes and their prevention, remain a topic of much concern. This is reflected in the fact that the subject 'Oxidation processes in milk and dairy products' attracted no less than 19 contributions at the XIIth International Dairy Congress (1949); these fill some 150 pages of the published proceedings. Most of the contributions deal with specific aspects, but also included are some more general surveys by Ritter (302), and by Greenbank (303), who has also reviewed the topic exhaustively elsewhere (304). Another review has come from Hood (305).

In a survey of market milk in Utah during 1940–50, Larsen (306) found oxidized flavour development in no less than 14·5–37·5 % of the random samples. He experimented with several antioxidants, as did Nikkilä (307) whose paper contains data on the ascorbic and dehydroascorbic acid content of Finnish winter milk.

Krukovsky and his colleagues (308) have continued their studies of the role of ascorbic acid in the oxidation of milk, cream and butter subjected to a variety of treatments, and have measured the losses of fat-soluble vitamins which occur during oxidative spoilage. They have made wide use of a test based on emulsification of the fat under investigation with vitamin C-free skim milk. Krukovsky, Loosli & Whiting (309) have found that the tendency of milk to produce oxidized flavours decreases when the tocopherol content of the milk fat is increased by feeding tocopherol to the cow. Reinart (310) has given extensive data on the tocopherol content of butter showing that the natural concentration in milk fat is too low to exert any significant antioxidative effects in butter. Added synthetic tocopherols proved to be rather weak antioxidants.

Sjöström & Larsson (311, 312) have given details of preparing an effective antioxidant for milk, cream and butter by heating whey in the absence of air to about 90° C., alkali being added to maintain a pH of 11. Stull, Herreid & Tracy (313) have presented further evidence of the value of nordihydroguaiaretic acid in preventing oxidative spoilage during storage of unsweetened frozen cream. The same authors (314) have shown the efficacy of very low concentrations of this acid in suppressing copper-induced oxidized flavour development in milk. The acid was one of eight aromatic hydroxy compounds tested by Swartling (315) for their antioxidative properties during storage of butter at 13 and −18° C. for short periods. It gave rise to an off-flavour when present in concentrations in which it was effective as an antioxidant. Nordihydroguaiaretic acid is also included in a study of antioxidants by Tollenaar (316) mainly devoted to the esters of gallic acid, and their effect on dry butterfat and dried whole milk. Certain flavones and flavone derivatives have been shown by Richardson, El-Rafey & Long (317) to be effective antioxidants for butterfat.

The importance of oxidative changes within the fat phase of dairy products has been stressed by Loftus-Hills (318). Holm & Wode (319) followed autoxidation in butterfat and vegetable oil by spectrophotometric measurements on suitably prepared extracts of the oxidation products. Specific absorption at 2300 Å. was linearly related to peroxide values for winter butter, but for summer butter this only applied after an initial lag during which the absorption changed but little. This lag is ascribed to the higher concentration of conjugated diethenoids in summer butter. The value and limitations of the peroxide test as a criterion of the degree of fat spoilage has been

discussed by Holm (320). Extensive experiments on the proportions of saturated, unsaturated and highly unsaturated fatty acids in the fat of Swedish creamery butter have led Reinart (321) to conclude that autoxidation depends largely on the content of highly unsaturated fatty acids in the C_{20}–C_{22} series, as these are strong pro-oxidants. Hillig & Ahlmann (322) have observed that the water insoluble fatty acids, defined as the difference between total acidity and the sum of lactic and volatile acids, increase markedly in deteriorating cream and are transferred to the butter made from such cream. Reinart (323) has demonstrated that diacetyl has pro-oxidative properties, but according to Pette (324) diacetyl has no adverse effect on butter stored for several months at $-10°$ C. Barnicoat (325) has tried to improve the keeping quality of butter by adding various substances during manufacture. Addition of a citrate buffer met with some success.

Lea & Smith (326) have summarized British work on gas-packing, high temperature preheating and the use of ethylgallate as practical means of preventing autoxidation of the fat in whole-milk powder. According to Wright & Greenbank (327), the addition of ascorbic acid to milk prior to its conversion into whole-milk powder increases the keeping properties of the nitrogen-packed product on storage at 37° C. The best results were obtained with 20 mg. ascorbic acid per l. of milk, the largest quantity used. In a study of factors affecting the browning of whole-milk powder, Tarassuk & Jack (328) suggest that browning without flavour deterioration occurs because the available oxygen might be taken up preferentially in the browning reaction. Workers from Urbana have shown (329, 330, 331) that the factor responsible for stale flavour in dried whole milk is associated with the fat, and may be isolated by a method based on steam distillation under reduced pressure.

Stine, Loos & Daume (332) have presented experimental evidence of the importance of keeping the copper content of Cheddar cheese below 2 p.p.m. Apart from other faults, cheese with a higher copper concentration tended to develop oxidized or metallic flavours. Hood (333) has discussed earlier work on flavour defects in Canadian Cheddar cheese.

Mulder and his colleagues (334, 335) have presented preliminary reports about the effect of variations in the pH of cream on the development of off-flavours in butter.

Tarassuk & Jack (336) have pointed out that lipolytic flavour arises from processes, such as homogenization or agitation of warm milk, that disrupt the membrane surrounding the fat globules in milk. Weinstein & Trout (337) homogenized milk from individual Ayrshire, Brown Swiss, Guernsey, Jersey and Friesian cows. Irrespective of breed, rancidity developed almost without exception on storage. Deterioration in the fat phase is, according to Musset, Patton & Dahle (338), at least partly responsible for flavour defects in freshly made whole-milk powder. Experiments after fractional crystallization of the butterfat showed that flavour defects develop rapidly in the liquid fractions.

The mechanism underlying the parallel development of cooked flavour and browning in sterilized evaporated milk has been discussed by Tarassuk (339). A report on weed taints, and the plants from which they originate, has come from Australia (340).

V. Analysis of milk and milk products

(*a*) *Milk*

Fat

The scientific committee responsible for the standardization of methods for the analysis of milk in the Netherlands has issued (341) a detailed specification of the apparatus and technique for the determination of the percentage of fat in whole milk by the Gerber method. In general it agrees with the specification of the British Standards Institution. During the period under review there has been a resurgence of interest in the accuracy of the Gerber test for milk as assessed by comparison with the more lengthy Röse-Gottlieb method, and it has been claimed by several workers (342, 343, 344, 345, 346) that the Gerber results are high by 2–3 % and represent the g. of fat per 100 ml. and not per 100 g. of the milk. This may be met, it is suggested, by reducing

appropriately the volume delivered by the milk pipette. Radema & Mulder(346) stress the fact that the Gerber test is empirical: they found its results (G) to correlate with Röse-Gottlieb results ($R.G.$) according to the equation $G = 1{\cdot}04\ R.G. - 0{\cdot}07$. The calibration of Gerber butyrometers for the estimation of fat in milk, cream, butter, cheese and powdered milk has been discussed by Fort(347). A burette for the rapid checking of the calibration of Gerber butyrometers has been devised by Dolby(348). It has a long capillary jet which passes through the graduated portion of the butyrometer into the bulb, and thus avoids splashing of mercury and inclusion of air bubbles.

The various procedures for the Babcock test for fat in milk, as specified in the regulations of the different States of the U.S.A., have been collected and critically discussed(349). The lack of uniformity is emphasized and it is recommended that a standard procedure be agreed and approved by the American Dairy Science Association. Likewise, a committee of the Victorian Division of the Australian Society of Dairy Technology(350) has considered the Australian regulations governing the Babcock test inadequate and made recommendations for improving the reproducibility and accuracy. With the same object, Herreid, Burgwald, Herrington & Jack(351) advise the elimination of the upper meniscus by the addition of coloured glymol and enlarging the milk pipette to hold 18·04 ml. at 35–36° C. Trout, Brunner & Lucas(352) found that when the Babcock test was applied to milk reconstituted from powder it gave low results.

Schain(353) was the first to explore the use of detergents in place of sulphuric acid in the Babcock test: he used two detergent solutions – 7 ml. of solution 'A', a mixture of Oil Red O in isopropyl alcohol, with the non-ionic detergent polyoxyethylene sorbitan monolaurate in ethyl alcohol, and 13 ml. of solution 'B' containing the anionic dioctyl sodium phosphate. His results agreed well with those of the ordinary Babcock test. Gershenfeld & Ucko(354) confirmed this and modified the technique to advantage, reducing the volumes of the two solutions used and combining these in one single solution. The modified test has been successfully applied to milk, cream, butter, cheese and ice-cream by them and by Gershenfeld & Rosenthal(355).

A full description of the Röse-Gottlieb method for the determination of the fat content of milk has been given by Radema & Mulder(356), who favour the use of Mojonnier extraction tubes. Muers & House(357) have stressed that the presence of peroxides in the ether used in the Röse-Gottlieb method may cause high results, and they recommend treatment of the ether by storing over a moist zinc-copper couple. Goiffon(358) has suggested a method for the estimation of fat in milk based on the photometric measurement of the opacity of a dispersion of the extracted fat in acetone and sodium carbonate solution. Heinemann & Rohr(359) have proposed a routine micro-method for fat in skim milk and skim-milk powder involving measuring the area of a mono-layer formed by transferring 0·007–0·008 ml. of ether extract to a prepared surface on 0·2 % acetic acid.

Protein

In the search for quicker and simpler techniques for the determination of the total nitrogen content of milk, Mogensen(360) has described a convenient application of well-known principles to the digestion, distillation and titration of about 1 mg. amounts of nitrogen, and Hetrick & Whitney(361) have employed nesslerization of the digested sample, using gum acacia to prevent turbidity. Kofranyi(362) has suggested further simplification, and reports that results accurate within 3 % may be obtained by measuring the ammonia liberated when milk is steam-distilled directly with sodium hydroxide and barium chloride under specified standard conditions for exactly 10 min.

By virtue of its extreme simplicity, the formol titration for the estimation of milk protein has continued to attract investigators(363, 364, 365, 366, 367). The assessments of its relative accuracy vary appreciably, but it remains—as much earlier literature showed—only an approximate method. Goiffon(368, 369) has developed a colorimetric method for the milk protein, using Folin and Ciocalteu's reagent to react with the tyrosine and tryptophan, and a standard casein solution for comparison.

Lactose and lactic acid

Malpress & Morrison (370) have evolved a colorimetric method for estimating lactose based on its reaction with methylamine and giving results within 5 % of the true value. Horowitz, Davidson, Howard & Reithel (371) applied this method to mammary gland preparations and found it the most convenient of three methods tested for the purpose. Hites & Ackerson (372) have revived the determination of lactose in a deproteinized milk filtrate by heating with alkaline ferricyanide and titrating the excess iodometrically, and have given full details of their technique. Perry & Doan (373) have evolved a colorimetric method for the simultaneous determination of lactose and sucrose in milk and milk products involving the reduction of picric acid.

For the colorimetric determination of lactic acid in milk and milk products, Davidson (374) has adapted Barker & Summerson's method for lactic acid in blood. The protein and lactose are removed with copper sulphate and lime, the lactic acid is oxidized to acetaldehyde in sulphuric acid, and a purple colour is developed with *p*-hydroxydiphenyl. When 20–400 mg. of lactic acid were added to fresh milk recoveries of 96–103 % were obtained.

Kruisheer (375) has investigated the methods of Soxhlet-Henkel, Dornic, Thörner, Mann and Van Norman for the estimation of the titratable acidity of milk, and has noted the effects of varying the dilution and the concentration of indicator. He recommends a standardized procedure for use in Holland. Foschini (376) has shown the effect of a varying carbon dioxide content on the titratable acidity, and suggests that milk should be warmed under reduced pressure, and shaken to expel the gas, before titration. Bryant (377) has recommended a new solvent for the phenolphthalein indicator for the titratable acidity estimation; 1 g. of indicator is dissolved in 60 ml. of ethylene glycol monomethyl ether and diluted with 40 ml. of water. This solution shows none of the usual deposit on storage, and gives results identical with the ethyl alcohol solution. Roeder (378, 379) has reported further on the use of his proprietary indicator solution 'Frigidol' for estimating the pH of milk and assessing its freshness on receipt at the factory. Talce (380) has used the test in mastitis control work to detect abnormal quarters.

Minerals

Kägi (381) has discussed the advantages and disadvantages of the preliminary wet digestion procedure (with nitric and perchloric acids), as compared with dry ashing, for the determination of minerals in milk and milk products, and given advice on the avoidance of explosions with the acids.

The increasing use of flame photometry is reflected by the appearance of papers (382, 383, 384) describing the application of this technique to the determination of calcium, sodium and potassium in milk. Johnston, Gellman & Strom (385) have compared three methods for measuring the iron content of milk and suggested an improved method for the wet ashing of the sample. Hostettler, Schilt & Hänni (386) have described the application of a mercurimetric method to the direct determination of chloride in milk, butter and cheese, using diphenylcarbazone as indicator. Pyne & Ryan (387), by taking precautions to titrate the milk and its whey to the same pH value of 8·65, have improved the accuracy of the determination of the colloidal phosphate of milk.

Miscellaneous

Much improved lactometer jars for use in the routine estimation of the density of milk have been designed—in glass by Humphriss (388), and in copper by Martin (389). Both types make the operation quicker and cleaner, as the thermometer remains *in situ*, the overflow of milk is collected, and the jar is conveniently emptied from its base. Roeder (390) has prepared a table for correcting to 15° C. the lactometer readings taken for whey at temperatures of from 10 to 30° C.; and Kothavalla, Anantakrishnan & Paul (391) have extended the table of corrections for milk to cover the range of 50–110° F. for use with the milk of Indian cows and buffaloes.

Babad & Shtrikman (392) have evolved a method for the determination of citric acid in milk, a yellow colour being developed by treating a trichloroacetic acid filtrate with acetic anhydride and pyridine. Täufel (393) has reported values for citric acid in milk varying with the method of

preparation of the milk filtrate and the age of the samples. Wright & Anderson(394) have confirmed that their test for the addition of specified hypochlorites to milk is sensitive to 8 p.p.m. There is little reduction in sensitivity until the titratable acidity of average milk reaches 0·185 %.

Crowe, Jenness & Coulter(395) have examined Chapman & McFarlane's ferricyanide procedure for measuring the reducing capacity of milk. After encountering difficulties, particularly with milk powders of high reducing capacity, they have presented modifications which have given more satisfactory results: the pH has been raised to 6·6, the temperature of reaction lowered to 50° C., and the method has been calibrated in terms both of ferricyanide reduced and of ascorbic acid or cysteine oxidized. The study of simplified systems of milk constituents showed that some of the reducing substances produced by the heat treatment of liquid milk, and the ageing of dried milk, are formed from lactose and from protein-lactose interaction. Patton & Josephson(396) have made observations on the application of the nitroprusside test to heated milk. They found that this test for sulphydryl groups is best carried out at below 20° C., and described suitable dye standards for matching the colour. When skim milk, rennet whey, dialysed skim milk, and dialysed skim milk plus lactose were heated the intensity of the nitroprusside reaction increased with temperature up to 93·3° C. Further heating at this temperature caused a fall in intensity with skim milk and dialysed skim milk plus lactose, but little change with the others, indicating that loss of sulphydryl on prolonged heating is due to the combined action of casein and lactose.

Duggan(397) has described a method for the determination of free tryptophane in milk, cream and butter, and O'Moore(398) a rapid qualitative diffusion test for the detection of acetone in milk from cows suffering from ketosis. Bennet(399) has reported collaborative work by eight chemists on four methods of detecting formaldehyde in milk: the Hehner-Fulton (sulphuric acid and bromine) and chromotropic acid tests were the most satisfactory, and the latter, being highly specific, is recommended.

(*b*) *Butter and butterfat*

The Association of Official Agricultural Chemists has sponsored further search for a simple, yet satisfactory, procedure for the preparation of butter samples for analysis, and Meuron(400) has reported on a mechanical stirrer method applied to butter at 25° C. The Association has also pursued the study of the free water-insoluble fatty acids and butyric acid in cream and butter in view of the importance of these substances as an index of decomposition. Hillig(401) has described a method for the determination of the water-insoluble acids present in the free state or as inorganic salts in the fat or aqueous phases; and Hillig, Lepper & Patterson(402) have shown the increase in these acids (and in free butyric and propionic acids) in the cream, and the butter made therefrom, when samples of cream from various producers were held at summer temperature for periods of up to eleven days. Later, Hillig(403) reported similar collaborative work on butter, and Ramsey & Hess(404) reported a collaborative study of the A.O.A.C. method for the determination of the acetic, propionic and butyric acids in a mixture of acids by partition chromatography. The results suggested that the method is sufficiently accurate for the investigation of the decomposition of foods containing butterfat.

In preliminary work on the determination of the fully saturated glycerides in New Zealand butterfat, Ting(405) has found that with the present method, depending on oxidation of the unsaturated components by permanganate in a boiling acetone solution of the fat and subsequent separation and purification of the residue, there are difficulties in the purification stage, and he stresses the need for a new line of approach. For the determination of linoleic acid in butterfat, Schaffer & Holm(406) have measured the absorptions of the alkali salts of the fatty acids from butterfat in ultraviolet light of from 224 to 274 mμ, and from the degrees of absorption, and the specific absorption coefficient of diene and triene conjugations at 234 and 268 mμ, have calculated the linoleic acid content. This ranged from 2·11 to 2·40 %.

To provide a routine method for the determination of diacetyl in butter, den Herder(407) has modified the well-known Prill and Hammer method, using a stream of carbon dioxide instead of steam distillation to transfer the diacetyl from the melted butter to the hydroxylamine solution

in which the final red colour is developed. Pien(408) has improved the method of preparation of diamino-benzidrine, which now remains stable and white, and he is able to determine the diacetyl in butter by titration in a few minutes.

Jax(409) has investigated the use of the semi-automatic Bradender oven (designed for the estimation of moisture in cereals) for measuring the moisture content of butter, cheese, and dried and condensed milk. He concludes that the apparatus is suitable for this purpose, giving results which compare favourably with those of other quick methods, and from his experience suggests times and temperatures of heating for the various products. Dobinsky(410) has described a method of measuring the moisture content of butter based on its dielectric constant, and the automatic application of the method to the butter leaving the Fritz continuous butter-making machine.

Perrin, Lightfoot & Moir(411) have improved Moir's convenient filtration method for the determination of iron and copper in butter; modifications now permit the colorimetric determination of iron with thiocyanate and copper with carbamate to be made on the same sample. Preliminary notes are given on the determination of iron and copper in cheese and dried milk. Den Herder & Krol(412), also, have described routine colorimetric methods for iron, copper and manganese in butter, using the butter serum. Coleman, Thompson & Branum(413) have discussed, with special reference to copper in butterfat, the difficulty of dry-ashing fatty materials without loss of trace metals in the vapours, and have emphasized the limitations of wet digestion methods. They allow a weighed amount of fat to drop at the rate of one drop per 5 sec. into a Pyrex crucible containing a plug of glass wool and kept at a dull red heat.

Williams(414) has simplified the well-known method of Bolton, Richmond and Revis for the calculation, from the Reichert, Polenske and Kirschner values, of the proportions of butterfat, coconut oil and palm kernel oil in mixtures of these fats. Felman & Lepper(415) have described a rapid test to distinguish oleomargarine from butter: 2 ml. of the clarified fat are mixed with 2 ml. of a 2:1 mixture of ethyl and isoamyl alcohols, the mixture is heated until clear, and then cooled slowly. The temperature at which turbidity first occurs is noted. For butter and margarine samples this critical temperature was within the range 42–53 and 66–75° C. respectively. Neseni(416) has given a method for the separate estimation of nitrogen as total protein, casein, or soluble protein in butter.

(*c*) *Cheese*

Horwitz & Knudsen(417) have reported collaborative work on the sampling and determination of fat and moisture in cheese, and on the agreement in results obtained between laboratories and workers in a given laboratory. Several papers have dealt with rapid methods for the determination of the moisture content of cheese. Shemin & Wagner(418) add powdered calcium carbide to finely grated cheese, heat for 5 min., with stirring, in a boiling water bath, and take the loss in weight. They claim that the results compare well with those obtained by drying in a vacuum oven. Kumetat & Demmler(419) have also used calcium carbide, and have adapted the Fischer titration procedure to cheese (and butter) with good results. Kosikowsky & Dahlberg(420) have successfully used a semi-automatic drying oven for moisture in cheese and cheese products, and Péter(421) has described a quick method for moisture in curd. Hostettler(422) has compared and discussed the results of three commonly used oven-drying procedures for cheese moisture. The Roeder test for fat in cheese—an adaptation of the Gerber method, but using stannous chloride in hydrochloric acid as protein solvent—has been investigated by Bernaerts(423) who finds that the high results are due to the formation of amyl ether and amyl esters of fatty acids.

A method for estimating the degree of protein breakdown in cheese during ripening, using the formol titration, has been presented by Mogensen(424). The cheese is emulsified with sodium citrate, paracasein is precipitated at its iso-electric point (pH 4·4) with acid, and the formal titration of the filtrate at pH 8·3 and its nitrogen content by the Kjeldahl method are measured. Kosikowsky & Dahlberg(425) have made several improvements in, and have adapted for routine analysis, the earlier method of Bellamy *et al.* for the determination of tyramine. Hiscox & Berridge(426) have published a note on the use of paper partition chromatography for the

identification of the volatile fatty acids in cheese distillates or culture fluids. With bromcresol green as indicator, and ammonia or ethylamine to control the ionization of the fatty acids, the latter appear as blue areas on a greenish yellow background after 1 hr.

(*d*) *Dried milk, etc.*

Of the various arbitrary procedures that have been proposed for the estimation of the 'solubility' of roller-dried milk powder, the majority have failed to receive general approval, mainly because they were not simple enough or did not readily yield reproducible results. A method recently developed by Parsons(427), however, and based on the solubility of the solids-not-fat, should meet these requirements. Jaton(428), discussing the solubility of milk powder, has suggested an index of 'dissolution' to indicate the faculty of the powder to disperse as individual particles in water without mechanical aid, in contrast to the solubility resulting from 'dissolution' plus mechanical aid.

A reagent for the rapid and convenient detection of fat oxidation in spray-dried whole-milk powder has been developed by Dowden(429); it is prepared from dimethyl *p*-phenylenediamine hydrochloride, water, acetone and α-naphthol. When one drop of the reagent is added to a little of the powder a blue colour develops when the powder contains oxidized fat. Lea(430) has applied the ferricyanide method of Chapman & McFarlane to the estimation of reducing groups in milk powder. The increased reducing power on storage, as shown by this method, is attributable to reaction between the protein and sugar. Thiel, Loftus Hills & Scharp(431) have designed a special apparatus for the routine estimation of oxygen in tins of milk powder. The apparatus (which is of metal except for the absorption bulbs) punctures the tin, withdraws a measured volume of the gas therein, and circulates it through chromous sulphate in sulphuric acid.

Kruisheer(432), and Kruisheer and his colleagues(433), have tried several methods for the detection and determination of neutralizing agents and lactic acid in milk powder, pointing out the difficulty of demonstrating the addition of small amounts of sodium bicarbonate. Whitman & Tracy(434) have studied the ash alkalinity of buttermilks from sweet and neutralized creams. All samples from cream with 0·3 % or more of acidity neutralized with bicarbonate gave ash alkalinity values of 150 or above. Certain other neutralizers gave the same result with 0·2 % acidity. Horwitz(435) has supported the use of the sodium-chloride ratio for the detection of sodium salt neutralizer in skim-milk powder.

A convenient method for the titration of the free fatty acids of dried milk, cream and other dairy products in an 85 % ethanol extract has been evolved by Hollender, Rao & Sommer(436). Miller, Zimmerman & Oberg(437) have compared vacuum and steam distillation for determining the volatile acidity of evaporated milk. Choi and his co-workers(438), continuing their studies of moisture in dried-milk products, have evolved a toluene distillation method for determining the water of crystallization of α-lactose in dried whey. The method takes advantage of their earlier observations on the unimolecular nature of the dehydration of α-lactose hydrate. They have also given(439) a solubility method for the determination of α- and β-lactose in dried milk.

A valuable contribution on the determination of total solids in condensed whey, and the mother liquor left after the removal of lactose, has been made by Muers & Murphy(440). The determination presents difficulties owing to the complex composition of these substances and their low pH, and finding no published information, the authors tried various possible procedures. Only an oven-drying method proved suitable, and the method finally adopted (to prevent charring and the retention of lactic acid without appreciable loss of ammonia) involves the use of a sand support to facilitate the removal of moisture, the addition of powder calcium carbonate as neutralizer, and drying at 80° C. under reduced pressure.

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PART II. RHEOLOGY

Homogenized milk

Caffyn(1) has made a careful study of the viscosity and the viscosity-temperature coefficient of homogenized milk. The estimated accuracy of 1% is good for such a material. The usual plot of log viscosity versus reciprocal of absolute temperature does not give a single straight line; there seems to be an inflexion at about 40° C., probably caused by the melting of crystalline fat particles. At temperatures below 60° C., repeated shearing lowers viscosity: at higher temperatures viscosity rises. This rise, which occurs even in fat-free milk, is ascribed to changes in the protein. The initial fall at lower temperatures is caused by changes in the fat and does not occur in fat-free milk.

Sweetened condensed milk

Webb(2) and Webb & Hufnagel(3) discuss the effects of varying fore-warming time, temperatures, etc., on the viscosity of sweetened condensed milk. Logarithmic relations are established between storage temperature and viscosity and also the time taken to reach a specified viscosity. Storage time is linearly related to viscosity. Torsell, Sandberg & Thureson(4) measured viscosity and conductivity during concentration and found simple relationships between total solids, temperatures and viscosity but not conductivity. Reiner, Scott Blair & Hawley(5) have reported that, when a vertical rod is rotated in some over-aged sweetened condensed milk samples, the milk climbs up the rod ('Weissenberg effect'). It is suggested that this is dependent on some degree of protein denaturation.

Cream

Mohr & Wellm(6) have measured the temperature coefficient of the 'viscosity' (consistency) of cream, which is found to be very large. In concentrated creams, the globules are tightly packed and non-spherical in shape.

Butter

The same authors and Mohr & Schulz(7) describe a number of methods for measuring the consistency of butter, notably a sectilometer (cutting with a wire) and a cone penetrometer. In later papers, Mohr & Häsing(8) describe a machine which imitates the spreading of butter on bread. The force required to spread under standard conditions correlates fairly well with sectility data. The force required to drive a perforated plate through the butter is said to measure oiliness (Schmierigwerdens). The bending of beams of butter under gravity is also measured. This work is carried somewhat further by Mohr, Kaufmann & Koenen(9), who load beams of butter with weights.

Mulder(10) has studied the influence of churning temperature and cream fat content on butter consistency. Churning at 9° C. gave a harder butter than churning at 15° C. Fat content had no influence on hardness. The texture was markedly affected by churning time. Mulder has also summarized his work on butter in a lecture(11) and in a paper(12) given at the International Dairy Congress. Tverdokhleb(13) discusses faults in butter consistency and the effects of variations in salt- and moisture-contents. He compares 'quasi-viscosities' determined with a penetrometer with microscopic appearance. Csiszár(14) describes a penetrometer consisting of a steel rod with a rounded end which is dropped from a standard height on to the butter sample.

Cheese

Hansson & Hietaranta(15) and Hansson, Sjöström & Samuelsson(16, 17) discuss the effects of pasteurization and cooling on the elasticity of rennet curd, describing a grooved cylinder torsion wire rheometer for determining elasticity. The effects of variations in pH, $CaCl_2$ content, lactic acid, fat content, etc., are also discussed. The 'elasticity' is defined as the ratio of the angle through which the torsion head is turned to that through which the cylinder moves, minus one. Anderson & Weckel(18) have measured the so-called 'curd-tension' of skim-milk curd adjusted to

a number of specified fat contents. Except at the lowest fat content (0·5 %), heating to 170° F. for 30 min. lowered curd tension.

By far the largest body of rheological dairy research has been done on cheese. Koestler has extended his work described in the last Review in a number of papers (19, 20). Mulder & Radema (21) study the possibility of making cheese without the use of heat; the necessity for this arose in Holland during the war. It was found possible to make Edam cheese at 20° C. by adding substantial quantities of $CaCl_2$ and HCl to the milk. Whitehead (22) discusses firmness and smoothness of cheese as separate factors. He stresses the importance of an adequate manufacturing routine, but believes also that rheological tests would be helpful. He has investigated the effects of large variations in the fat content of the milk but concludes that the casein content is more important than fat content. High casein content makes for a dry curd. The caseins from Jersey and Friesian milks are markedly different. Davidov & Barabanshchikov (23) discuss 'objective methods for evaluating physical properties of cheese'. They use a Höppler Consistometer (24) (without acknowledgement), forcing a sphere through the cheese and comparing (*a*) control with pasteurized milk, (*b*) pasteurized milk with milk treated with 20 g. $CaCl_2$ per 100 kg., (*c*) also with 50 g. $CaCl_2$ and (*d*) also with 80 g. $CaCl_2$. The curd is also compressed by a piston into a cylinder at the end of which is an orifice through which the curd is extruded. Elasticities are also measured. The cheese is of Dutch type. It is concluded that the effects of pasteurization on plastic and elastic deformability can be offset by fairly large additions of $CaCl_2$ ($\sim$50 g. per 100 kg.). Kazantsev (25) measures the elasticity and consistency* of Altai cheese. The curd is compressed by a plate within a gauze container. The forces needed to give, first, elastic displacements and, later, to force the curd through the gauze are recorded on a dynamometer. The container consists of two parts hinged together. By this means the force required to break the curd can also be measured.

Hadland (26) has made a detailed study of the use of the sphere hardness tester devised by Scott Blair and his colleagues at Reading, for testing Norwegian cheese and whey-cheese (made from condensed whey). He concludes that the instrument, which he re-names 'the John Bull Indicator', gives excellent reproducibility and is of considerable practical importance. The total deformations (under arbitrary constant load) are correlated with total solid contents. The effects of repeated loadings on the same spot on the cheese are also studied.

Scott Blair and his colleagues have continued the work described in the last Review, especially in relation to the ripening of cheese in store. A summary is given by Scott Blair & Baron (27). The general principles involved in the application of physical methods to the dairy industry are discussed by Scott Blair (28) and by Harper (29). Scott Blair & Baron (30) describe a method by which the load on a sphere hardness tester can be increased to compensate for the increasing area of contact with the cheese. In this way, indentation can be measured under approximately constant stress.

The main body of this work during the period under review is described in a series of papers by Baron (31), Harper & Baron (32) and Baron & Harper (33, 34). Three main experiments are described, following the manufacture and ripening of Cheddar cheese during the 1947 season and also the ripening of Cheshire cheese during the two consecutive years 1949, 1950.

In the experiment on Cheddar, the effects of manufacturing variations on the properties of the 'green' (young) cheese are most clearly seen after 14 days. High fat content tends to produce a soft cheese and high solids-not-fat a tough cheese, at least when ripe. A 'soft cut' produces a springy type of cheese: a 'firm-cut' curd gives a firm and dry cheese. High acidity at milling produces a soft cheese with little 'spring'.

The first experiment was done on eleven Cheshire cheese of varying quality, studied in detail by rheological and subjective testing during a ripening period of two months. The curves showing the changes in various mechanical properties as the cheese ages reflect, and to some extent predict, the keeping quality of the cheese. In the later and much larger experiments some fourteen tests were carried out on about 200 cheese in the store and the data were submitted to a multiple

* The author uses a Russian word which means 'stickiness', but it is clear that this is not what he is really measuring.

factor analysis (see below). It is concluded that objective (rheological) tests alone 'cannot adequately predict all the important characteristics of Cheshire cheese, but must be used in conjunction with subjective assessments'. A study of the specifically subjective aspects of judging Cheshire cheese is given by Harper & Baron (35). The professional grader shows no unusual degree of consistency in discriminating between cheese. If asked to compare specifically for 'firmness', his discrimination is negligible. This is not, perhaps, surprising, since he does not regard 'firmness', as distinct from the wider concept of 'body', as of importance. Non-experts fairly quickly improve their powers of discrimination with practice. A further and more general discussion on the psychological aspects of craftsmanship is given by Harper (36).

Although it may be necessary to do fairly large numbers of tests on every sample of cheese, the results of the tests being intercorrelated, it may well be possible to describe their interrelationship in terms of a much smaller number of independent sources of variation, or 'factors'.

Scott Blair proposed to apply the method of multiple-factor analysis, much used in the handling of psychological test data, to rheological and subjective data for cheese, and a preliminary analysis was made by Harper & Baron (37). The same authors (38) performed an analysis on the main body of data from one of the experiments on about 200 Cheshire cheese (described above). Three important independent sources of variation were detected. The first is closely related to surface hardness (sphere indenter) and the second to subjectively assessed crumbliness. The third remains unidentified. Mocquot, following a study of the bound water in Gruyère cheese (39), co-operated with Scott Blair & Baron to use the method developed at Reading for determining rheological properties of curd, especially at the pitching point, and of finished cheese (40). With but minor modifications, the technique devised for English Cheddar and Cheshire were successfully applied to Gruyère. The sphere indenter gives data which enable the ripening quality of very young cheese to be foretold; and the tests, being easily reproducible, avoid the uncertainties inherent in subjective judgements. Mocquot, Scott Blair, Baron & Jeandot (41) describe the same series of experiments in French. Scott Blair, in examining Gruyère cheese in a French store, was struck by the peculiar orientation and shape of cracks ('Lainures') even when the holes ('yeux') appeared spherical. In co-operation with Reiner, an engineer-rheologist from Haifa, Israel, a short paper was published suggesting the types of stresses likely to cause such conditions within the cheese and the probable moisture and temperature conditions producing them (42).

Cervical secretions

Glover & Scott Blair have continued work on the rheological properties of bovine cervical secretions. A new emptying and filling micro-capillary viscometer was designed in which the rate of intrusion and extrusion is maintained constant, the varying pressure needed for this purpose being plotted against the lengths of the column in the capillary. The central part of these curves was found to be nearly linear, but the slopes varied very widely during the sexual cycle and in pregnancy. Marked dips in the curves plotting these slopes against date indicate oestrus, and although these dips persist to a less marked degree well into pregnancy, a suitable averaging of the points gives a curve which rises characteristically, in normal cows, a few weeks after the onset of pregnancy. Certain types of infertility and subfertility also show characteristic curves (see Glover & Scott Blair (43)). (Very similar results had been obtained from secretions from the human cervix—see Clift, Glover & Scott Blair (44).) An attempt by Scott Blair (45) to use a rotating cylinder apparatus for bovine secretions, though yielding results of rheological interest, did not add anything to our knowledge of the relationships between rheological properties and physiological conditions.

The much simpler instrument known as the oestroscope, designed at Reading (see earlier Reviews) has been tested on a fairly large scale by Herman and his colleagues in U.S.A. (for a general account see Raps (46)). This instrument measures the flow-elasticity of the secretions. Herman & Horton (47) discuss the rheological properties of cervical secretions in relation to sperm permeability. Working with eighty Holstein (Friesian), Jersey and Guernsey cows, they find that

the 'viscosity' (consistency) of the secretions increases at oestrus, the penetrability is at a maximum 6–12 hr. after the start of oestrus, the volume decreases during oestrus, while the number of leucocytes increases. There are periodic variations in the vaginal temperature during the oestrous cycle and slight variations in pH. Roark & Herman(48) give curves showing a progressive fall in flow-elasticity during the hours of oestrus. Surface tensions and pH were also measured. An *in vitro* technique for following the penetration of secretions by sperm is described. De Vuyst & Vervack(49) confirm a maximum flow-elasticity at oestrus. Using the oestroscope on over 300 cows, they concluded that the value of the oestroscope reading is not correlated with the chance of successful insemination. Bostico(50) studies the chemical characteristics of dried mucus and quotes Callisti (probably Illari & Callisti(51)) as having measured viscosities of, presumably, diluted samples of secretion.

Though not strictly within the realm of rheology, Rothschild's work(52) on changes in the electrical impedance as a measure of the activity of ram and bull sperm should be mentioned in this Review.

General

Ogston, Stanier, Toms & Strawbridge(53) have studied synovial fluid in a rotation viscometer. The material shows shear elasticity and yields less under rapidly applied stresses than would a truly viscous system. This may account for its value in protecting neighbouring membranes from shock.

Ogston & Stanier(54) have studied the action of hyaluronic acid on synovial fluid. Viscosities were measured in an Ostwald capillary viscometer and anomalous viscosities in a Couette rotation viscometer. Relative viscosity is approximately linearly related to glucosamine concentration. Intrinsic viscosities $[\eta]$ are calculated: $[\eta]$ is defined as the limiting specific viscosity divided by concentration, extrapolated to zero concentration). Using Simha's method, the axial ratio of the molecules is calculated from $[\eta]$ to be ~ 590. Sedimentation and diffusion give ~ 60. This discrepancy suggests a sponge-like structure of rod-like particles. The hyaluronic acid-protein complex has a molecular weight $\sim 10^6$. The particles are rods 200μm. in length $\times 3{\cdot}3\mu$m. in diameter. The main conclusion is that hyaluronic acid occurs in a definite compound with protein or other material, which can be separated by ultra-filtration.

Finally, three papers should be mentioned which, while dealing with foodstuffs other than dairy products, nevertheless include the latter. Scott Blair(55) has written a general summary of work on the rheology of foodstuffs and Morse, Davis & Jack(56) have studied the effect of addition of non-fat milk solids in increasing the consistency of flour pastes as measured with a Stormer rotation viscometer and a Bloom gelometer. The second part of the paper is concerned with various other food products, sauces, custards, etc., for which a falling ball viscometer is also used.

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